

**COMPARATIVE EFFECTS OF HYDROCORTISONE, DEXAMETHASONE
AND METHYLPREDNISOLONE ON HEALING OF EXPERIMENTAL
CANINE WOUNDS MANAGED CLINICALLY.**

BY

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MARCH, 2011

DECLARATION

I declare that the work in the thesis entitled “Comparative Effects of Hydrocortisone, Dexamethasone and Methylprednisolone on Healing of Experimental Canine Wounds Managed Clinically” has been performed by me in the Department of Veterinary Surgery and Medicine of Ahmadu Bello University Zaria under the supervision of Profs. A. Z. Hassan, E. O. Gyang and M, Y. Fatihu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any Tertiary Institution.

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CERTIFICATION

This thesis entitled “COMPARATIVE EFFECT OF HYDROCORTISONE, DEXAMETHASONE AND METHYLPREDNISOLONE ON HEALING OF EXPERIMENTAL CANINE WOUNDS MANAGED CLINICALLY” by Japheth J. Kalang, meets the regulations governing the award of the Degree of Masters of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the Lord Most High, Jehovah Jireh.

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ABSTRACT

Twelve dogs divided into four groups of three each were screened to determine the effects of corticosteroids on experimentally created surgical wounds. Under general anaesthesia and using standard technique, 2 × 3 cm and 5 cm excisional and incisional wounds were created respectively on either side of the dorsum of experimental dogs. Dogs in groups I served as the control, group II had hydrocortisone (1mg/kg IM), group III had dexamethasone (0.5 milligram (mg)/kg IM) and group IV had methylprednisolone (1mg/kg IM). Dogs were treated for 5 days post surgery. Heparinized blood samples were routinely taken. Biopsies of surgical wounds were taken on days 3, 7, 14, 18 and 21 post-surgery and studied histopathologically. Grossly wound contraction in control group I dogs was comparable to that in group II dogs that had hydrocortisone in excisional wounds. For incisional wounds dogs in control group I had best contraction. Inflammatory reaction was abundant in the control group I dogs and least in group IV dogs that had methylprednisolone. Scar tissue formation was best in group IV dogs that had methylprednisolone, although the difference was of no statistical significance. It was concluded that; methylprednisolone had the best arrangement of arrangement of collagen, early epithelialisation and the thinnest scar. This could be recommended for facial, ocular and other cosmetic surgery; dexamethasolone produced the thickest scar and therefore could be recommended for use in wounds where high strength is required like herniorraphy and; dexamethasone and methylprednisolone-treated dogs showed absence of itching during wound healing when compared to the control and hydrocortisone-treated dogs. Moreso, clinical use of corticosteroids in the management of surgical wound had no significant effect on the quality of healing.

Finally, investigations are needed at the molecular and micromolecular levels to ascertain the effects of these steroids on the quality of wound healing.

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CHAPTER 1

INTRODUCTION

1.1 Preamble

A wound is defined as a disruption of the anatomical, physiological and cellular continuity of normal body surface (Pascoe, 1982), while others refer to wound as, any form of bodily injury often sustained from an external source resulting in the separation of skin, mucous membrane, or an organ surface (Hassan and Hassan, 2003). Surgical process involves creation of wound as well as managing wounds. To be surgically successful, wounds should heal properly with minimum scarring, preservation of tissue, return of function and minimum healing time (John, 1974; Milne, 2008). In recent times, wound management has been of great concern in both human and animal health. Improper wound management results to (a) the increased duration of hospital isolation (b) increased cost of treatment (c) loss of function (d) death of patients (John, 1974).

Wound healing commences immediately after injury mediated by cytokines and chemokines. The duration of wound healing process varies with wound type, management, microbiologic and physiologic factors (Kumar, 2002; Kahn, 2005). However, for wounds to heal they must undergo the following processes; inflammation, debridement, repair and maturation (Kahn, 2005). These processes have been known to be influenced by several intrinsic and extrinsic factors among which are steroids.

Steroids are chemicals that encompass the natural glucocorticoids, cortisone and hydrocortisone hormones while the synthetic steroids include the following in an ascending order of potency; prednisolone, methylprednisolone, triamcinolone, bethamethasone and dexamethasone (Boden, 1999). Steroids exert both metabolic and hormonal effects by influencing gluconeogenesis, viscosity and lubrication by joint fluid; they increase blood flow to the skin and reduce sebaceous gland secretion, as well as the function and formation of connective tissue, thus depressing scarring (Hassan and Egege, 2004). Steroids sensitize vascular muscle to nor-adrenaline, maintain normal capillary permeability, myocardial contractility and control excessive inflammatory response. They also limit edema formation, therefore allowing better penetration of antibiotics. The use of steroids for days is rarely troublesome (some authors however have varied opinion) but dosing for more than a week at inflammatory-suppressing dosage carries the possibility of suppressing pituitary-adrenal responsiveness. The major physiological role for steroids is that of increasing the ability of the body to resist stress (Hemingwa, 1980; John,1985; Brander *et al.*,1991). The prophylactic use of steroids in pediatric strabismus repair shows a satisfactory wound healing without any infection or delay. Moreso, steroids were found to decrease pain, swelling and vomiting after molar tooth extraction (Rashmi *et al.*, 2005). Although inflammatory processes were considered as defence mechanisms, they are also directly responsible for many of the symptoms and complications of numerous diseases (Ruy, 1961; Madden, 1972).

1.2 Justification

The general impression about steroids with wound healing is that they are drugs to be avoided. However, the major physiological function of steroids is to increase the ability of

the body to resist stress (Brown, 2008; Zukerman and Infinger, 2008); stabilize cellular function (Radostits *et al.*, 1999) and reduce inflammatory reactions (Milne, 2008). Thus Goforth and Gudas (1980) reported that steroids are useful to minimise postoperative edema and pain. Subsequently Busti, *et al.*, (2005) recommended the use of anti-inflammatory drugs in perioperative settings. Though the use of steroids is discouraged in infectious diseases, steroids were seen to give favourable results (Drulle, 1997; Fitch and Van De Beek, 2008). Moreover, conflicting reports occur on the effect of steroids on wound healing; like steroids may delay wound healing (Kamillosan, 1975; Pongvarin, 2004; Milne, 2008), while Peacock and Vanwinkel, (1976) reported that, steroids appear not to have any effect on wound healing. Glowania *et al.*, (1987) and Nasser *et al.*, (2008) reported that, steroids were used to speed-up wound healing. The use of steroids with increase in protein intake also enhances wound healing (Salcido, 1999). Hence, this study was undertaken to validate the above assertions as they relate to the three steroids being used on our local dogs.

1.3 Aim and Objectives

The aim of this study was to individually and comparatively assess the effects of hydrocortisone, dexamethasone and methylprednisolone on experimental canine wounds, with the following objectives:-

1. To evaluate the effects of hydrocortisone, dexamethasone and methylprednisolone on the inflammatory process, epithelialisation, granulation tissue formation, collagenisation, wound contraction and remodeling of experimental canine wounds.

2. To assess the rate (duration) and quality of wound healing with special interest in scar tissue formation following the administration of hydrocortisone, dexamethasone and methylprednisolone at therapeutic dose.
3. Determine the various side effects of hydrocortisone, dexamethasone and methylprednisolone used in the study individually and to compare their outcome.

1.4 Hypothesis

The study hypothesis was that, the three steroids (hydrocortisone, dexamethasone and methylprednisolone) used in the study do not have any significant effects on wound healing managed clinically at the therapeutic dose and duration used.

CHAPTER 2

LITERATURE REVIEW

2.1 Wound

A wound is a disruption in the normal structure of an organ or tissue caused by an external agent, with disruption of the normal continuity of structures (organ or tissue) (Martin, 2002; Blood *et al.*, 2007). The disruption could be deep, extending even to the bone as described by Thomas, *et al.*, (2000).

2.2 Wound Healing

2.2.1 Introduction

Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue. Wound healing involves a complex and dynamic but superbly orchestrated series of events leading to the repair of injured tissues. A completely healed wound is one that has returned to its normal anatomical structure, function and appearance, without drainage or dressing required (Golsen, 1989; Anon^h, 2009; Anon^a, 2010). All wounds follow roughly the same healing process. Understanding the processes involved in wound healing is essential to making well-founded decision in the management

of wounds. The wound healing process involves four different phases: Inflammation, debridement, repair and maturation (Blood, 2007; Anon^b, 2009).

2.2.2 Inflammatory phase

The inflammatory phase starts with the injury itself (Anon^b, 2009). It is characterized by hemostasis and vasodilatation (Linehan *et al.*, 2005). During wounding, collagens from the endothelial cells are exposed. The exposed collagen then activates the clotting cascade initiating the inflammatory phase. The damaged cell membranes release thromboxane A₂ and prostaglandin 2- α , which are potent vasoconstrictors which attempts to control bleeding by constricting the microvascular vessels with fibrin, fibronectin, vitronectin, von Willebrand factor, platelets and thromboplastin making a clot. This initial response helps to limit hemorrhage. After a short period, capillary vasodilatation occur secondary to local histamine release, and the inflammatory cells are able to migrate into the wound bed (Ueno *et al.*, 2006; Gabriel *et al.*, 2009). The inflammation phase, leads to infiltration of polymorphonuclear leucocytes to the wound site within twenty four to forty eight hours. Polymorphonuclear leucocyte activity usually ceases within a few days of wounding once contaminating bacteria have been cleared. Monocytes on arrival at the wound site become tissue macrophages (Thomas *et al.*, 2000; Anon^h, 2009). Macrophages are the most important cells present in the later stages of the inflammatory process (48-72 hours) and appear to act as the key regulatory cells for repair as they release cytokines and growth factors into the wound, recruiting fibroblasts, keratinocytes and endothelial cells to repair the damaged blood vessels. The lymphocyte is the last cell type to enter the wound during the inflammatory phase (greater than seventy two hours post-wounding). Lymphocyte

may also be involved in collagen and extracellular matrix remodelling (Thomas *et al.*, 2000; Anon^h, 2009; Anon^e, 2010).

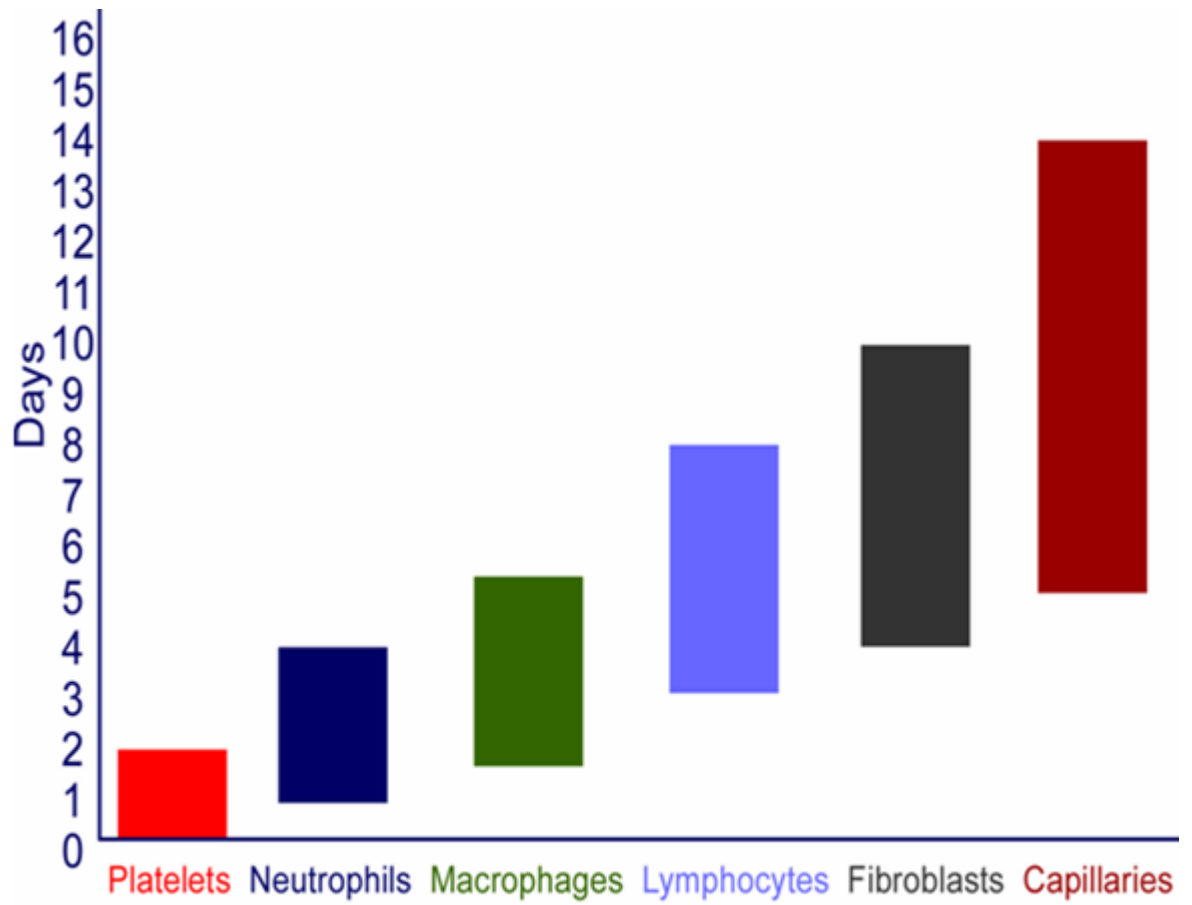


Fig. 1.1 Blood cells involved in wound healing and their time of activity

Source: Sabiston *et al.*, (2001).

With time, the density of macrophages and fibroblasts is reduced by apoptosis (Gabriel *et al.*, 2009). The timeline for cell migration in a normal wound healing process at the wound site is predictable (Fig. 1.1).

Platelets release platelet-derived growth factor and transforming growth factor- β to attract neutrophils and macrophages. Neutrophils scavenge for bacteria and foreign debris while macrophages emit growth factors to attract fibroblasts (Gabriel *et al.*, 2009).

2.2.3 Debridement phase

Debridement means the removal of dead tissue. The white blood cells clean the wound of dead tissue, debris and bacteria. Monocytes on reaching the wound transform to macrophages which do not only phagocytise dead tissue, debris and bacteria but also releases proteolytic enzymes such as collagenase that can debride tissue. Debridement can be accomplished in an autolytic manner, meaning the wound itself is encouraged to do this task by the use of dressings (Anon^h, 2009; Anon^a, 2010). The low oxygen tension between the wound and bandages results in a low pH, this, in turn, deters bacteria growth, favours collagen synthesis, enhances angiogenesis, and attracts white blood cells. In addition such bandages are comfortable to the patient. As healing progresses, these bandages can be left in place longer between changes (Hunt, 2000; Swaim, 2010).

2.2.4 Proliferative phase

The proliferative phase which is also called the repair phase starts at about day three after injury and last for about 3 weeks (or longer) depending on the severity of the wound. It overlaps with the inflammatory phase. The most important cells in this phase of wound healing are the fibroblast, which peaks approximately seven days from injury and are responsible for initiating angiogenesis, epithelialization, and collagen synthesis (Thomas *et al.*, 2000; Gabriel *et al.*, 2009). In the repair phase, there are three important aspects to be considered; wound contraction, granulation tissue formation and epithelialisation.

2.2.4.1 Wound contraction

Wound contraction is defined as the centripetal movement of the edges of a full thickness wound in order to facilitate closure of the defect. Fibroblasts at the stroma of the wound differentiate into myofibroblasts, causing tissue contraction during this phase of wound healing. The wound gradually contracts and is covered by a layer of skin (Gabriel *et al.*, 2009; Anon^b, 2009). Contraction is maximal between 5th and 15th day after wounding and is mediated to a great extent by the myofibroblast. Connections between granulation tissue and myofibroblasts mediate the forces of contraction in a united fashion throughout the open wound (Gabbiani, 1972). Wound contraction occurs when the underlying contractile connective tissue shrinks in size to bring the wound margins closer together. Contraction thus occurs through the interactions between fibroblasts and the surrounding extracellular matrix. Wound contraction does not only achieve reduction in the size of the wound but also subsequent organisation of the early collagen depositions which is initially highly disorganised (Anon^b, 2009).

2.2.4.2 Granulation tissue formation

Granulation tissue is so called because of the pink granular appearance of numerous capillaries that invade the wound stroma (Hunt *et al.*, 2010). The process of forming new blood vessels occurs concurrently during all phases of the healing process as platelets during this phase attract macrophages and granulocytes to promote angiogenesis. As collagen accumulates in the granulation tissue however, the density of blood vessels diminishes and disturbance of this dynamic process may influence the development of chronic wounds. Fibroblasts synthesise collagen to provide strength and integrity for all tissues in the body: Once within the wound environment, fibroblasts proliferate and start to construct the new extracellular matrix (Golsen, 1989). Collagen is recognised as the foundation of wound extracellular matrix. Granulation tissue is mainly made up of proliferating fibroblasts, capillaries and tissue macrophages in a matrix of collagen, glycosaminoglycans including hyaluronan, and the glycoproteins fibronectin and tenascin. Granulation tissue formation is evident as early as 48 hours after wounding and by 96 hours post-wounding fibroblasts become the predominant cell at the wound stroma since they are critical cells in the formation of granulation tissue. Fibroblast does not only synthesise collagen from its rough endoplasmic reticulum but also produces elastin which is important in wound remodelling. Shortly after wounding, resident skin fibroblasts and perivascular mesenchymal cells differentiate into a phenotypically different cell the “Myofibroblast”. Rough endoplasmic reticulum in the fibroblast is the site of collagen synthesis (Golsen, 1989; Peacock and Kelman, 1990). Granulation tissue formed in this phase of wound healing is particularly important in wound healing by secondary intention. When collagen

synthesis and breakdown become equal, the next phase of wound healing starts (Gabriel *et al.*, 2009).

2.2.4.3 Epithelialisation

A layer of epidermal cells starts to migrate from the wound edges (i.e epiboly) within a few hours of wounding. These cells lose their normal firm attachments to the underlying dermis, allowing them to migrate in a leap-frog fashion across the provisional matrix. Further epidermal cell movement is halted by contact inhibition (Thomas *et al.*, 2000; Anon^h, 2009). The initial event in epithelialisation is the migration of undamaged epidermal cells from the wound margins, if the defect is superficial enough, that is not affecting the dermis. Proliferation is maximal at forty eight to seventy two hours post wounding and is reflected by a seventeen-fold increase in epidermal cell mitosis and epithelial hyperplasia at the wound edges (Loning *et al.*, 2004). The epithelium seeks a plane of migration with a critical humidity. The plane of movement of epidermal cells is determined in part by the water content of the wound bed. Open, desiccated, superficial wound epithelialises much more slowly than occluded wound. Occlusive and semioclusive dressings optimally promote re-epithelisation postoperatively (Peacock *et al.*, 1990; Hunt *et al.*, 2010). Keratinocytes assist in the process of re-epithelisation by producing certain factors like: fibronectin, collagenases, plasminogen activator, neutral proteases and collagen. These factors promote adhesion of keratinocytes and assist in their guidance across the wound base, they are also important in debridement of devitalised tissue (Peacock and Kelman, 1990). Epithelialization occurs from the basement membrane, that is, if the basement membrane is intact, however, if the basement membrane is not intact, epithelialization

occurs from the wound edges (Gabriel *et al.*, 2009). Cells travel about 3cm from the point of origin in all directions during wound healing (Fishman, 2009).

2.2.5 Remodelling phase

Remodelling phase in wound healing lasts about two years. This phase is characterized by decrease in neo-angiogenesis, increase in collagen production and increase in collagen breakdown until it equals normal skin ratio. During this phase, collagen crosslinks and reorganizes along lines of tension, giving added strength to about 70-80% of the strength of the uninjured tissue. During this phase vascularity decreases, producing a less hyperemic and more cosmetically appealing wound (healed wound) as reported by Gabriel *et al.*, (2009) and Anon^b (2009). The initial new collagen formed by fibroblast (collagen III) are being replaced by collagen I and II during this phase of wound healing; changing the shape of the wound and increasing the strength of tissues in the wound area. The body's ability to heal during this stage is diminished in the elderly due to some physiological changes like reduced proliferative capacity of the epidermal cells and flattened dermo-epidermal junction (Stuart and Patricia, 2010). As remodelling of the wound continues, matrix metalloproteinases activity decreases and tissue inhibitors of metalloproteinases activity increases to inhibit protein breakdown (Thomas *et al.*, 2000; Anon^h, 2009). With continued remodelling, the outgrowth of capillaries is halted, blood flow to the area is reduced and metabolic activity in the area declines. An acellular avascular scar, is the final result of an acute wound healing process.

2.3 Classification of Wounds

According to Anon^e, (2010) wounds can be classified as follows:

Stage 1:- Stage 1 wounds are characterized by redness or discoloration, warmth and swelling or hardness. The epidermal lining is not completely penetrated.

Stage 2:- Stage 2 wounds partially penetrate the skin not penetrating the basement membrane.

Stage 3:- Stage 3 wounds describe full skin thickness wound.

Stage 4:- Stage 4 wounds involve damage to undermining adjacent tissue muscle or even the bone.

2.4 Wound Management

2.4.1 Wound cleansing

Antiseptics can be used for the preparation of the patient, personnel and some surgical instruments. Moreso, they are used postoperatively to prevent infection or on a healing infected wound, thus giving the body a chance to repair itself. Most accepted wound-cleansing solutions have been demonstrated to be toxic to fibroblasts and lymphocytes. These solutions include povidone-iodine, acetic acid, iodophor, hydrogen peroxide and Dakin's solution (sodium hypochlorite). The only acceptable wound-cleansing solution that is not detrimental to cells is normal saline solution (0.9% sodium chloride, or salt in water). Normal saline solution effectively removes contaminants and has the same salt concentration as the fluid in cells. In addition, normal saline is inexpensive and readily available with close to zero chances of hypersensitivity reactions. Dislodging the fragile granulation tissue or skin that is forming in the wound bed during wound dressing will

delay wound healing (Thomas *et al.*, 2000). Other wound cleansing agents include, alcohol which is another antiseptic employed but has the disadvantage of slow bacteriocidal effect. However, chlorhexidine has a wide spectrum of antibacterial activity with good residual activity and low systemic absorption and toxicity; neither does it induce bacterial resistance (Harvey, 1980).

2.4.2 Wound treatment

No single dressing or wound treatment is suitable for all types of wounds. Often a number of different types of dressings will be used during the healing process of a single wound. The following functions are expected to be performed by wound dressing and treatments: maintain a moist environment at the wound/dressing interface; absorb excess exudate without leakage to the surface of the dressing; provide thermal insulation and mechanical protection; provide bacterial protection; allow gaseous and fluid exchange; absorb wound odour; be non-adherent to the wound and easily removed without trauma; provide some debridement action (remove dead tissue and/or foreign particles); sterile, non-toxic, non-allergenic and non-sensitising (Ngan, 2010).

Drugs that affect inflammation and local immune responses are necessary for proper wound healing in the perioperative setting as stated by Busti, *et al.*, (2005). Furthermore, an understanding of fetal wound healing may lead to therapeutic strategies to help avert scarring and fibrosis. Healing without scar will have a tremendous impact on both medical and surgical practice. Probably in the future, gene therapy will become the standard treatment of enhancing wound healing (Stadelmann *et al.*, 1998).

2.4.3 Wound healing gels

Common gel used in wound dressing and treatment include: penicillin gel; gentamicin gel; chamomile gel and charmil® cream.

2.4.3.1 Penicilline gel

Penicillin gel has some antibacterial activities as it is an antibacterial drug which mainly acts on gram-positive bacteria but has the disadvantage of being inactive in the presence of the enzyme penicillinase and also cannot penetrate the thick coat of gram-negative bacteria (Meyer *et al.*, 1985).

2.4.3.2 Gentamacin gel

Gentamycin gel is also an antibacterial drug which is known for its effectiveness against gram-negative bacteria but has the limitation of being narrow spectrum which is specific against gram-negative bacteria (Grahame-Smith and Aronson, 1987).

2.4.3.3 Chamomile®

Chamomile® is also a topical gel preparation. Preparation of the herbal drug chamomile® was seen to be very effective when combined with corticosteroids and antihistamines. It has been used to speed-up wound healing in the elderly people with stasis ulcers caused by inadequate circulation as well as in people who had tattoos removed (Glowania *et al.*, 1987).

2.4.3.4 Charmil® gel

Charmil® gel is another common gel use in wound dressing and treatment which is a special combination of the extract of two herbs It doesn't contain any synthetic compound or antibiotic. It can be applied with great efficiency especially during hot season when external conditions enhance the proliferation of microbes and maggots on the surface of the skin and even on open wounds, charmil® can favourably be used. This gel is resistant to different kinds of weather, easy to use, completely absorbed and can be used to manage injuries and ailments of the skin. The gel also has an excellent insect-repellent feature with its characteristic smell (Anon^g, 2010).

2.4.4 Advances in wound managements

2.4.4.1 Hyperbaric oxygen

Hyperbaric oxygen therapy is used to treat or manage very serious wounds. The patient breathes 100% oxygen in a pressurized chamber for 90-120 minutes. The oxygen dissolves into the blood and is distributed throughout the body, providing extra oxygen to the cells attempting to heal the wound. This treatment has been found to increase the rate of collagen deposition, angiogenesis, and also microbial clearance. Moreso, oxygen therapy had been seen to stabilise wound area and then demonstrate a progressive reduction in wound area (Joseph and Christ, 2007).

2.4.4.2 Ultrasound

Ultrasound management or treatment uses mechanical vibration which is delivered at a frequency above the range of human hearing. Physical therapists report that covering the wound area with a hydrogel film and applying ultrasound during the inflammatory and proliferative stages stimulates the cells involved in wound healing and also warms the tissue, enhancing healing by improving circulation (Joseph, 2010).

2.4.4.3 Electrical stimulation

Electrical Stimulation mimics the body's own bio-electric system that influences wound healing by attracting repair cells, changing the permeability of cell membranes and therefore, affecting secretions and orienting cell structures. Electrical stimulation uses electrodes that are positioned around the wound area to emit some electrical charges. It can be used on most wounds during all phases of wound healing to support, speed, and even improve wound healing. The use of this therapy results in a smoother and thinner scar. (Sumano *et al.*, 2002).

2.4.4.4 Growth factors

Growth Factors are being studied intensively. These are biological substances that exert their influence by causing cellular growth and proliferation. They are considered as the main factors that regulates wound healing process (Anon⁸, 2010).

2.4.4.5. Gene therapy

Molecular genetic approach involves synthesis of genetically modified cells to deliver the desired growth factor in a time-regulated and locally restricted manner to the wound site. In contrast to many differentiated cell phenotypes, stem cells are potentially permanent residents of the wound site, and transducing them will therefore have the most lasting therapeutic effect. In addition, the function of genetically modified cells might be strengthened by implanting them in biomaterial scaffolds that promote cell adhesion, proliferation, migration and differentiation and that provide the basis for recreating a regenerative rather than a reparative wound environment (Petrie, *et al.*,2003; Mackool, *et al.*,1998; Mast, *et al.*,1992).

2.5 Factors Affecting Wound Healing Process

2.5.1 Introduction

Recognizing and understanding factors affecting healing wound process may lead to improved clinical management of wounds. Once a more favourable environment has been achieved, like protecting the wound from drying, excessive inflammation, and cooling from evaporative heat loss, wound healing process can be greatly influenced (Hunt *et al.*, 2010).

2.5.2 Infection

Infection of a wound will slow the healing process. However, all wounds contain some level of bacteria that usually does not affect the healing process. The difference between contamination and colonization is the central point in wound infection since it has to do with the concentration of bacteria in the wound stroma. Signs of infection include red skin around the wound, discharge containing pus, swelling, warmth, foul odour and fever. If a

wound is infected it does not respond immediately to over-the-counter antibacterial creams. Moreso, anti-tetanus prophylaxis is essential in wounds but is seldom seen now because of antibiotic treatment and vaccinations. The elderly and individuals with reduced immunity are at risk of wound-related infections, with any type of wound even seemingly minor injuries (Thomas *et al.*, 2000; Anon^e, 2010). These factors are:-

2.5.3 Age

Some physiological effects with age that affect wound healing include: reduction in the turnover of keratinocytes in the epidermis; reduction in cell population within the dermis; and microscopically reduced dermal thickness; the rate of epithelialisation declines and also migration of capillary epithelial cells with age; the dermo-epidermal junction also flattens, reducing the proliferative capacity of the epidermal cells and leading to its atrophic appearance (Anon^f, 2010; Stuart and Patricia, 2010). Apart from their inability to proliferate, there is reduced vitamin D, collagen and moisture, sebum and sweat production. Fibroblasts show decreased motility with increased latent time and reduced responsiveness to growth factors. Fibronectin accumulate in the extracellular matrix environment due to an increase in the rate of synthesis and also alteration in the physical nature of the fibronectin which causes a decrease in its cell-adhesive properties, decrease in the number and increase in the size of elastin fibres, reducing the skin's flexibility and decrease in Langerhans cells, associated with immune system functions. Formation of granulation tissue in aged is delayed compared to young and middle-aged mice. The impaired formation of granulation tissue may be related to a decrease in fibroblast numbers and collagen density (Stuart and Patricia, 2010; Hunt *et al.*, 2010). Some authors

however reported that aging has no effect on collagen synthesis (Holt *et al.*, 1992; Zulkowski and Albrecht, 2003).

The process of cutaneous wound repair is controlled by growth factors. Growth factor and receptors were assessed within acute incisional wounds in an ageing mouse. A delay in appearance of platelet derived growth factor A and B isoforms, and platelet derived growth factor- α and - β receptors was evident with increasing animal age, paralleled by a similar finding for epidermal growth factor and epidermal growth factor receptor. Contrary to this findings, transforming growth factor- β 1 and 2 isoforms were increased at all time points in the wounds of younger animals (Gillian *et al.*,1997).

2.5.4 Nutrition

Nutrition plays an essential role in wound healing and needs to be considered as a fundamental part of wound management care practices. Wound causes a number of physiological changes in the body that can affect the healing process, including changes in energy, protein, carbohydrate, fat, vitamin and mineral metabolism. These effects can be pronounced even with a small wound (Woodward *et al.*, 2010). Good nutrition facilitates wound healing, whereas malnutrition delays, inhibits and complicates the wound healing process (Collier, 2010). Some essential nutritional components include, protein, carbohydrates and fat, and vitamins

2.5.4.1 Protein

Protein depletion can affect the rate and quality of wound healing as reported by Gray and Cooper, (2001), since there is an increase in demand for protein in the presence of a wound, as part of the inflammatory process and in the development of

granulation tissue. The main protein synthesised during the healing process is collagen, and the strength of the collagen determines the strength of the wound. Protein is lost via wound exudate and needs to be monitored especially if dressings are being changed frequently. In such cases, protein replacement should be considered since they are essential for the maintenance and repair of body tissue (Gray and Cooper 2001; Woodward *et al.*, 2010).

2.5.4.2 Carbohydrates and fats

As part of the healing process, cellular activity is fuelled by adenosine triphosphate (ATP) which is derived from glucose, providing the energy for the inflammatory response to occur. In the case of insufficient carbohydrate supply, the body breaks down protein to provide glucose for cellular activity (Gray and Cooper, 2001). The main demand for energy from a wound is for collagen synthesis. Caloric needs for healing increase according to increasing size and complexity of the wound. Importantly, adequate fats are needed to prevent the body using protein for energy (Woodward *et al.*, 2010).

The role of essential fatty acids in wound healing cannot be ignored as they are major components of cell membranes and are involved in the synthesis of new cells and therefore, fatty acid demand increases after injury (Collier, 2010).

2.5.4.3 Vitamins

Vitamin 'A' increases the inflammatory response in wounds, stimulating collagen synthesis. Vitamin 'A' is also involved in the cross-linking of collagen and the proliferation of epithelial

cells. It has also been shown that vitamin 'A' can restore wound healing impaired by long-term steroid therapy or by diabetes (Woodward *et al.*, 2010).

B-Complex vitamins are co-factors or co-enzymes in a number of metabolic functions involved in wound healing, particularly in the energy release from carbohydrates (Collier, 2010; Woodward *et al.*, 2010).

Vitamin 'C' plays an important role in collagen synthesis and subsequent crosslinking as well as the formation of new blood vessels (angiogenesis). Vitamin 'C' also has important antioxidant properties that help the immune system as well as increases the absorption of iron, which is important as a cofactor in collagen synthesis (Collier, 2010; Woodward *et al.*, 2010).

Vitamin 'K' is involved in the formation of thrombin, and its deficiency in the presence of wounds could lead to haematoma (Collier, 2010).

2.5.5 Hydration

Hydration is important in wound healing as dehydrated skin is less elastic, more fragile and more susceptible to breakdown. Dehydration will also reduce efficiency of blood circulation, which will impair the supply of oxygen and nutrients to the wound. One of the main risk factors for dehydration is poor oral water intake (Woodward *et al.*, 2010).

2.6 Steroids

2.6.1 Definition

The term 'steroid' is applied to a group of naturally occurring or synthetic fat-soluble organic compounds (lipids), whose structure is chemically based on a steroid nucleus, that is a hydrogenated cyclopentanoperhydrophenanthrene ring nucleus of seventeen carbon atoms arranged in four rings which is composed of three six member rings and one five member ring. The substitution of hydrogen in this ring results in different drugs activities (Anon^d, 2009). Many hormones, body constituents and drugs are steroids (Anon^c, 2009).

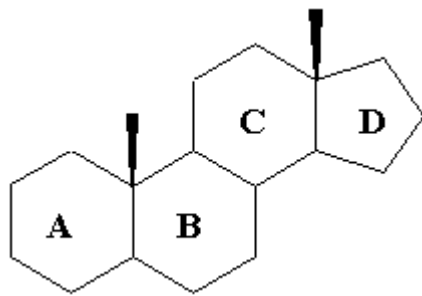


Fig.2.2 Basic structure of the steroid ring

Source: Anon^d, (2009)

2.6.2 Steroids and wounds

Since some podiatric surgeons as observed by Goforth and Gudas, (1980) use glucocorticosteroids to minimize postoperative edema and pain associated with traumatic procedures, the effects on wound healing should be understood as much as possible so that maximum results can be achieved with minimal risk to the patient despite some studies that indicate a detrimental wound-healing effect with the use of glucocorticosteroids. Corball *et al.*, (1985) recorded that there was a significant beneficial

effect from an alternate day steroid versus a daily steroid regime. It is well known that corticosteroids inhibit collagen synthesis, neo-capillary budding thereby retard wound healing when given prior to, or soon after, injury. Vitamin A may reverse this potentially serious side effect of corticosteroids while preserving their beneficial action. Recent work has suggested that this may be only when Vitamin A is given in toxic doses and only for a short period.

Wound healing was impaired by a single subcutaneous injection of 6 mg of methylprednisolone acetate, however, methylprednisolone treatment as observed by Woodward *et al.*, (2010) significantly decreased tumour growth factor- β levels in the wound fluid and hydroxyproline content of tissue. Steroids and retinoids have antagonistic effects on growth factors and collagen deposition. These effects can be relevant for treatment options in a clinical setting (Wicke *et al.*, 2000). For instance, increased collagen expression by human dermal fibroblasts was noted in response to the cardiogenic steroid marinobufagenin in a dose- and time-dependent fashion of up to eightfold increase in collagen synthesis (Nasser *et al.*, 2008).

Steroids interfere with inflammation, fibroblast proliferation, collagen synthesis and degradation, deposition of connective tissue ground substances, angiogenesis, wound contraction, and epithelialisation, consequently, causing dehiscence of surgical incisions, increased risk of wound infection and delayed healing of open wounds (Anstead, 1998). This is also supported by a work done by John *et al.*, (2010), where he studied a reproducible muscle contusion injury model in a rat. At day two, the corticosteroid group showed significant improvement in both twitch and tetanic strength relative to the

controls. At day seven, this effect was reversed and the corticosteroid muscles were significantly weaker than the muscles in the control group. At day fourteen, the muscles in the corticosteroid group were totally degenerated, with disorganized muscle fiber architecture. However, in the anabolic steroid group, their muscles were significantly stronger relative to muscles in the control group. Furthermore, androgen receptor, which is known to be pivotal to the action of male hormones such as testosterone, has been found to play a crucial role in the body's ability to heal. The receptor as reported by Ani, (2010) delays wound healing. This is a very interesting observation for people at the marginal end of health and the elderly, since, anabolic steroid therapy combined with increased protein intake has been successful in promoting weight gain, reversing catabolism, and increasing the rate of wound closure (Salcido, 1999).

Another beneficial use of steroids, since they are known to increase the number of circulating neutrophils is in patients that undergo total laryngectomy as neutropenia and neutrophil dysfunction, has been shown to play a role in poor healing (Thane *et al.*, 2010). Furthermore, Thane *et al.*, (1999) suggested that neutrophil dysfunction (which was observed) should be considered as predisposing factor to developing fistulas in patients with head and neck cancer.

Dexamethasone effectively decreases the incidence of nausea and vomiting with a single dose and was observed to significantly reduce collagenization, epithelization, and fibroblast content. The vascularity and the degree of inflammatory cells were more intense in the dexamethasone group compared with the control group. The white blood cell count was similar in the control and dexamethasone groups (Mahmut *et al.*, 2003). In females, this decline in the effectiveness of skin repair mechanisms follows menopause and a series

of clinical studies has identified estrogens as being an endogenous enhancer of the healing processes (Gilliver and Ashcroft, 2007).

2.6.3 Steroids and blood cell dynamics

Therapeutic doses of corticosteroids frequently induce eosinopenia; this could be as a result of these steroids (hydrocortisone, methylprednisolone and prednisone) leading to inhibition of eosinophil adherence and chemotaxis (Leonard *et al.*, 1981). Corticosteroids cause a decrease in the recirculation of blood cells from peripheral compartments (Jeffrey *et al.*, 2010; Kim *et al.*, 2010).

Glucocorticosteroid therapy results in an increase in the number of circulating neutrophils and a decrease in the number of eosinophils (Bjornson *et al.*, 1985; Haul, 2010; Gemzell *et al.*, 2010), this is as a result of steroid's ability to inhibit neutrophils cell death by apoptosis. However, suppression of apoptosis can be abolished by co-treatment with the protein synthesis inhibitor cycloheximide, like actinomycin D, polymyxin b and Roussel-Uclaf (RU)-486 (mifepristone), since corticosteroid regulation of neutrophil apoptosis depends on continuous stimulation of synthesis of a (protein) survival factor (Cox and Austin, 1997). Kothari and Saunders, (1961) observed that the resultant release of hydrocortisone and other glucocorticoids from the adrenal cortex causes a marked eosinopenia.

Administration of hydrocortisone decreases the absolute number of circulating lymphocytes and monocytes within 4-6 hrs but counts returned to normal by 24 hrs after administration (Anthony and David, 1974). Reinhart *et al.*, (2010) and Albert, (2010)

reported that corticosteroids induce a deficit in lymphocyte number and function, they also inhibit macrophage but not granulocyte exudation in rabbits. The function of the monocyte-macrophage system may be seriously impaired by corticosteroids. Furthermore, Shilov *et al.*, (2010) reported that hydrocortisone stimulates neutrophil phagocytic activity and decreases oxygen-dependent microbial activity of phagocytic cells. The administration of hydrocortisone led to depression of eosinophil phagocytosis and lesser decrease in monocyte phagocytic activity. Glucocorticoids characteristically induce eosinopenia in vivo and are effective for treating allergic and other eosinophilic disorders, dexamethasone, methylprednisolone, and hydrocortisone inhibited eosinophil survival in a dose -and -time -dependent manner. In contrast, estradiol and testosterone had no effect on eosinophil survival (Wallen *et al.*, 1991).

2.6.4 Physiological and clinical uses of steroids

2.6.4.1 Physiological role of steroids

The body naturally tend to contain stress, whether physical or neurological via an immediate and marked increase in adrenocorticotrophic hormone secretion. One possible reason for cortisol secretion is that the glucocorticoids causes rapid mobilization of amino acids and fat from their cellular stores, making them immediately available both for energy and for synthesis of other compounds including glucose needed by the different tissue of the body. When cortisol are secreted or injected into the body, they have two basic anti-inflammatory effects (1) they can block the early stage of inflammatory process before inflammation even

begins (2) if the inflammation already begins, it causes rapid resolution of the inflammation (Guyton and Hall, 2001).

Specifically steroids

(1) They stabilize the lysosomal membranes

(2) They decrease permeability of the capillaries

(3) They decrease both migration of white blood cell into the inflamed area and phagocytosis of the damaged cells

(4) They suppress the immune system causing decrease in lymphocyte production

(5) They lower fever mainly because they reduce the release of interleukin-1 from the white blood cells (Guyton and Hall, 2001).

2.6.4.2 Clinical uses of steroids

1. Wounds: when inflammation has already begun, the immediate effect of steroids is to block most of the factors responsible for the inflammatory reactions but in addition the rate of wound healing is enhanced. This could result from mobilisation of amino acids for repair of the damaged tissue and also gluconeogenesis to provide extra glucose in critical metabolic system (Guyton and Hall, 2001).

2. Toxaemia: steroids are used in toxaemia especially when shock is part of the pathogenesis. The rational for their use include organell- and –cell membrane stabilization, improved cellular metabolism and gluconeogenesis, improved

microcirculation, decreased production of endogenous toxin, leukocyte activation and degranulation (Radostits, 1999).

3. Anaphylaxis and anaphylactic shock: in anaphylaxis and anaphylactic shock, steroids are used to potentiate the effect of epinephrine (the primary drug of treatment) (Radostits, 1999); although the primary drugs for the treatment of allergy now is steroids.

4. Debilitating disease and the elderly: for people at the marginal end of health and the elderly. Anabolic steroid therapy combined with increased protein intake has been successful in promoting weight gain, reversing catabolism, and increasing the rate of wound healing (Salcido, 1999).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Experimental animals

Twelve local dogs between ages 12-18 months of both sexes were purchased and housed in the Small Animal Kennel of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria.

3.1.2 Drugs

Drugs used in this study include: steroids (dexamethasone, hydrocortisone and methylprednisolone); drugs for tick control (cypermethrin and ivermectin); anthelmintics (praziquantel); broad spectrum antibiotics (10% oxytetracycline); preanaesthetic agents (atropine sulphate and chlorpromazine hydrochloride); anaesthetic agents (thiopentone sodium and lidocaine); chlorhexidine (purit®) and methylated spirit.

3.1.3 Surgical materials

Wound template (exposed X-ray film); syringe and needle (1ml and 5ml syringes with 23 and 21 gauge needles respectively); Surgical materials (surgical gloves, scalpel blade, scalpel holder, needle holder, thumb forceps and haemostatic forceps, towel clamp, nylon as non-absorbable suture material and drape-set).



Plate I. Wound dressing materials used in the study

KEY

A= Disposable gloves size 7.5

B= Adhesive plaster

C= Crêpe bandage

D= Sterilised Gauze

E= Purit® (antiseptic)

F= Absorbent cotton

G= Dilute Purit® (antiseptic)

H= Charmil®

I= Thumb forceps

3.1.4 Dressing materials

Charmil® gel, gauze, absorbent cotton, polyethene bags, plaster, crêpe bandage, improvised Elizabethan collar and disposable gloves as shown in plate I.

3.1.5 Sample Materials

Formaldehyde (4%), tissue sample bottle, heparinized bottle, scaple blade and thumb forceps, lidocain and xylazine.

3.2 Methodology

3.2.1 Pre-surgical checks

The twelve dogs between ages 12-18 months (both sex) and weighing 10-18 kg, were housed in the kennel for two weeks in Ahmadu Bello University Veterinary Teaching Hospital Zaria for acclimatization. The dogs were physically examined, blood and faecal samples were taken for laboratory screening at the protozoology, clinical pathology and helminthology laboratories for haemoparasite, haemogram and helminthes respectively. The samples taken to protozoology laboratory were negative for all haemoparasites and the haemogram from the clinical pathology laboratory were all within normal range. However samples sent to helminthology laboratory were positive for tape worm and hook worms, the animals were treated with praziquantel at 7mg/kg and oxytetracycline antibiotics at 20mg/kg for secondary bacterial infection. At the end of conditioning and acclimatisation, samples were taken to the laboratories and were all negative for helminths and haemoparasites, with normal haemogram. Food and water was provided *ad libitum* throughout the period of experiment; six hours before surgery feeding and water was withdrawn. The work was done in three batches for each of the four groups represented. The first batch of the animals was between the months of July and August 2010, the second batch was between October- November and November- December 2010 for the third set of the animals. All pictures and data were labelled and documented during each animal evaluation.

3.2.2 Surgical preparations

Animals were shaved on the day of the surgery and taken to the surgical room. Following all aseptic procedures, the animals were placed on sternal recumbency and pre-anaesthetized with atropine sulphate at 0.02mg/kg and chlorpromazine hydrochloride at 0.5mg/kg intravenously (IV), they were finally anaesthetized with thiopentone sodium at

25mg/kg IV. The animals were scrubbed with 2% chlorhexidine solution then placed on the surgical table on sternal recumbency and rectangularly draped (Plate II).

3.2.3 Wounding procedure

Placing the sterilised wound template on the cranio-dorsal part of the animal, an indelible marker was used to mark a 2 x 3 cm area on the dorsum of the animal. About 5 cm away from the scapular and 3 cm from the vertebrae for the excisional wound on the left side; 15 cm from the excisional point, a 5 cm mark was made 3 cm away from the vertebrae on the left side of each animal. The marks on the right side were reversed to incisional wound 5 cm away from the scapular and 3cm from the vertebrae while the excisional wound was 15cm from the incisional wound. Plate II. shows the animal aseptically prepared and draped with the wounding sites marked ready for the wounding. This was done for all the dogs used in this study.

Using a sterile scalpel blade, the wounds were created and haemorrhage was controlled by application of sterilised gauze under digital pressure or haemostatic forceps. The wounds were then dressed using dry dressing. This dressing was done daily until absence of pus discharges and subsequently on alternate days to reduce interference with the healing process.

3.2.4 Treatments and sample collection

The animals were grouped randomly into four groups of 3 dogs each (Table 1). The dogs in the control group (group I) were not given any medication (steroids), the hydrocortisone

group (group II) which were given hydrocortisone at 1mg/kg body weight intramuscularly, the dexamethasone group (group III) were given dexamethasone at 0.5mg/kg intramuscularly and methylprednisolone group (group IV) were given methylprednisolone at 1mg/kg intramuscularly. The steroids were administered for 5 days post-wounding. Blood samples were collected in heparinized sample bottles using sterile needle and syringe for total protein concentration, Packed Cell Volume and differential leucocytes count in the clinical haematology laboratory of the Ahmadu Bello Veterinary Teaching Hospital, Zaria. Skin biopsy was taken for histopathological evaluation of the wounds on days 3, 7, 14, 18 and 21 post-wounding (Table 1). For the skin biopsy, the site was anaesthsized using lidocaine. A 2 cm skin biopsy was taken, 1cm into the wound and 1 cm of the surrounding apparently normal skin. The skin biopsy was fixed in sample bottles containing 20ml of 4% formaldehyde. The samples (skin biopsy) were trimmed and sectioned. The histological slides were prepared in the histological laboratory of the Human Anatomy Department. Each wound from which biopsy was taken was excluded from the study.

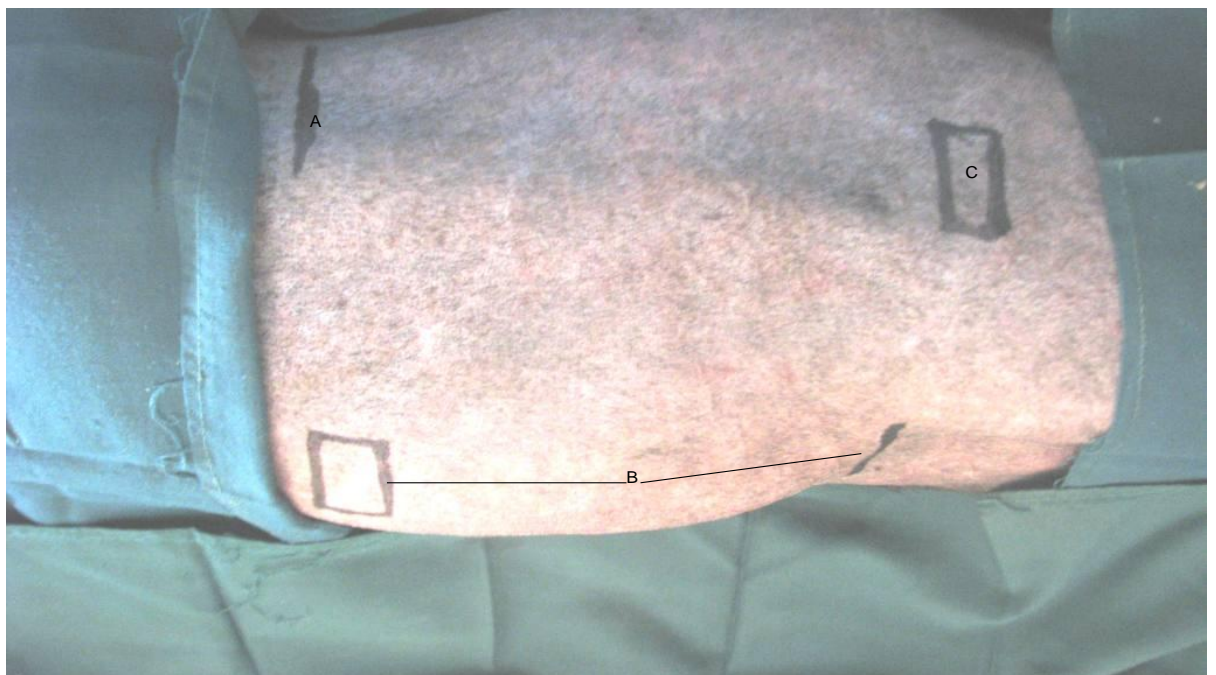


Plate II. Wounding site marked with an indelible marker and labelled

KEY

A = Incisional wound of 5cm

B = Distance between excisional and incisional wound 15cm

C = Excisional wound site of 2 x 3cm

Table 3.1. Experimental design.

Gp	Treatment	No. of dogs	Days of samples collection		Days for wound evaluation
			Blood sample	Skin biopsy	

I	No steroid given	3	0,3,5,7,14,18,21	3,7,14,18,21	0,3,5,7,12,14,17, 19,21,24,26,28
II	Hydro. 1mg/kg IM. 5/7	3	0,3,5,7,14,18,21	3,7,14,18,21	0,3,5,7,12,14,17, 19,21,24,26,28
III	Dexa. 0.5mg/kg IM. 5/7	3	0,3,5,7,14,18,21	3,7,14,18,21	0,3,5,7,12,14,17, 19,21,24,26,28
IV	Methyl. 1mg/kg IM. 5/7	3	0,3,5,7,14,18,21	3,7,14,18,21	0,3,5,7,12,14,17, 19,21,24,26,28

KEY:-

Gp= Group

No.= Number

IM= Intramuscular

Ctr= Control

Hydro= Hydrocortisone

Dexa= Dexamethasone

Methyl= Methylprednisolone

3.2.5 Post wounding care

Aseptic procedures were adhered to during post-operative wound dressing and sampling (plate III).

The wounds were cleaned three to four times at each dressing with sterilised gauze and dilute chlorhexidine (purit®) by gentle damping to avoid bleeding, this was done for all the wounds and all the groups. Wound dimensions were taken using measuring tape. Charmil® gel was then applied and subsequently covered with a sterile gauze, stabilized with an adhesive plaster and padded with cotton. The whole dressing was then held in place with crêpe bandage to avoid interference by the animal through scratching or licking.

3.2.6 Gross evaluation of the wounds

The wounds were assessed for epithelialisation; wound contraction was measured each day of dressing. The pus formation were also assessed as: mild that is pus present, moderate that is pus covered the wound stroma and high that is pus pouring off the wound stroma; granulation tissue formation was also assessed as mild that is slightly pinkish with still some brownish colouration, moderate that is slightly pinkish and high that is pink in colour.



Plate III. Dressing code for sampling and wound dressing.

KEY

A = Surgical cap

B = Face mask

C = Scrub suite

D = Sterile guaze

E = Excisional wound

F = Thumb forcep

G = Disposable gloove

3.2.7 Histological evaluation of wounds

Histological slides were prepared as described by Smith and Bruton, (1978). Slides were viewed at magnification of $\times 100$; the wounds were evaluated for severity of the inflammatory cells present as mild with few inflammatory cells, moderate and severe that is with diffused inflammatory cells respectively. The degree of edema was also evaluated and scored as mild, moderate and severe. Neovascularisation, connective tissue using elastin fibre, myofibroblast and collagen deposits were all assessed. Granulation tissue formation was also evaluated as low, moderate and high representing $\frac{1}{3}$, $\frac{2}{3}$ and complete granulation tissue formed over the wound area.

3.3 Documentation and analysis of result

Mean values of excisional wound area, incisional wound area, rate of epithelisation of excisional and incisional wounds were subjected to statistical analysis using One way Analysis of Variance (ANOVA) SPSS version 17 to determine the significance of the result at probability value of 0.05% (95% confidence interval); probability value less than 0.05 was considered significant ($p < 0.05$) . All the data were graphically represented, using line graph.

CHAPTER 4

RESULTS

4.1 Gross evaluation of wounds

Granulation tissue formation was visible in the four experimental groups by day 3 from the wound edges and completely became bright pink by day 14 post-wounding in all the groups. Pus formation was observed in all the groups by day 13 and ended by day 10 in the control and dexamethasone groups, however, pus persisted in hydrocortisone-treated group to day 12 post-wounding and day 14 post-wounding in the methylprednisolone-treated group.

Signs of itching occurred in the control and hydrocortisone treated group on days 3 and 8 post wounding respectively. However, this was not seen in the dexamethasone and methylprednisolone-treated groups. Hair growth after shaving occurred by day ten post-

wounding in the methylprednisolone and hydrocortisone-treated groups compared to the hair growth in the control and the dexamethasone-treated groups which were reduced.

The mean excisional wound area in ascending order of size, were control hydrocortisone, dexamethasone, and methylprednisolone treated group respectively. The mean excision wound dimensions and epithelialisation were presented in Fig. 4.1.1 and 4.1.2, appendix 1. and 3. respectively, while the mean incisional wound dimensions and epithelization were presented in Fig. 4.1.3 and 4.1.4, appendix 2. and 4. respectively.

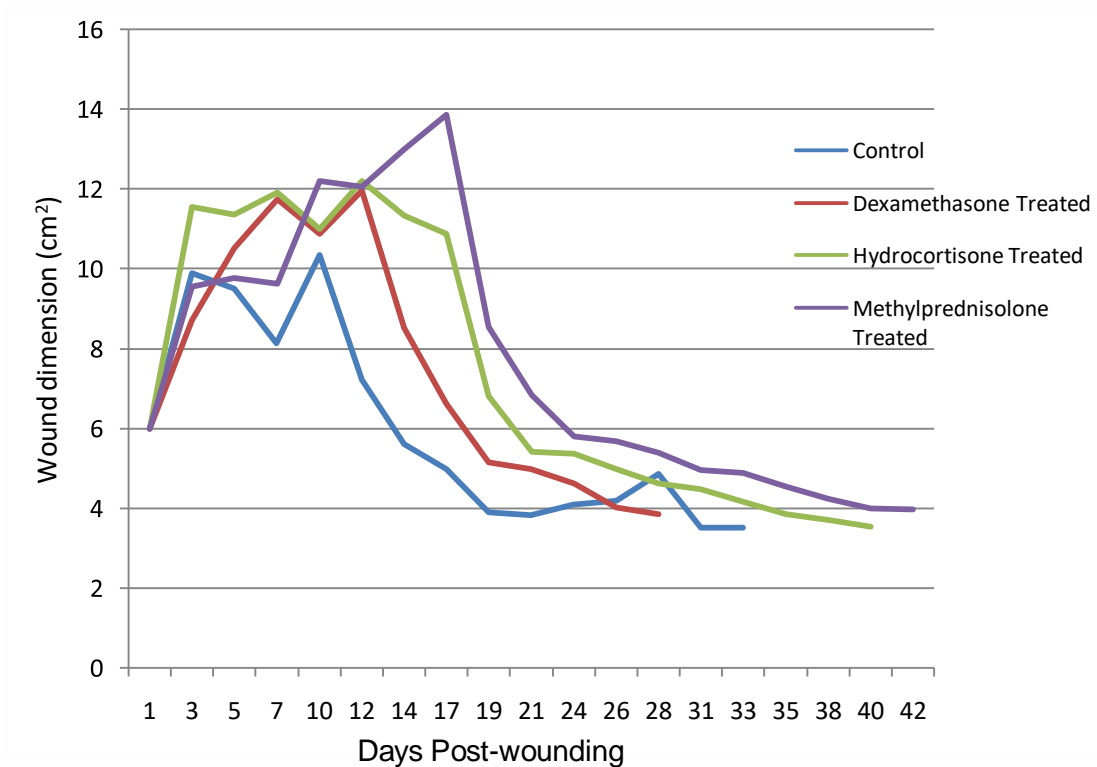


Fig.4.1.1 Mean excisional wound area (p-value = 0.499)

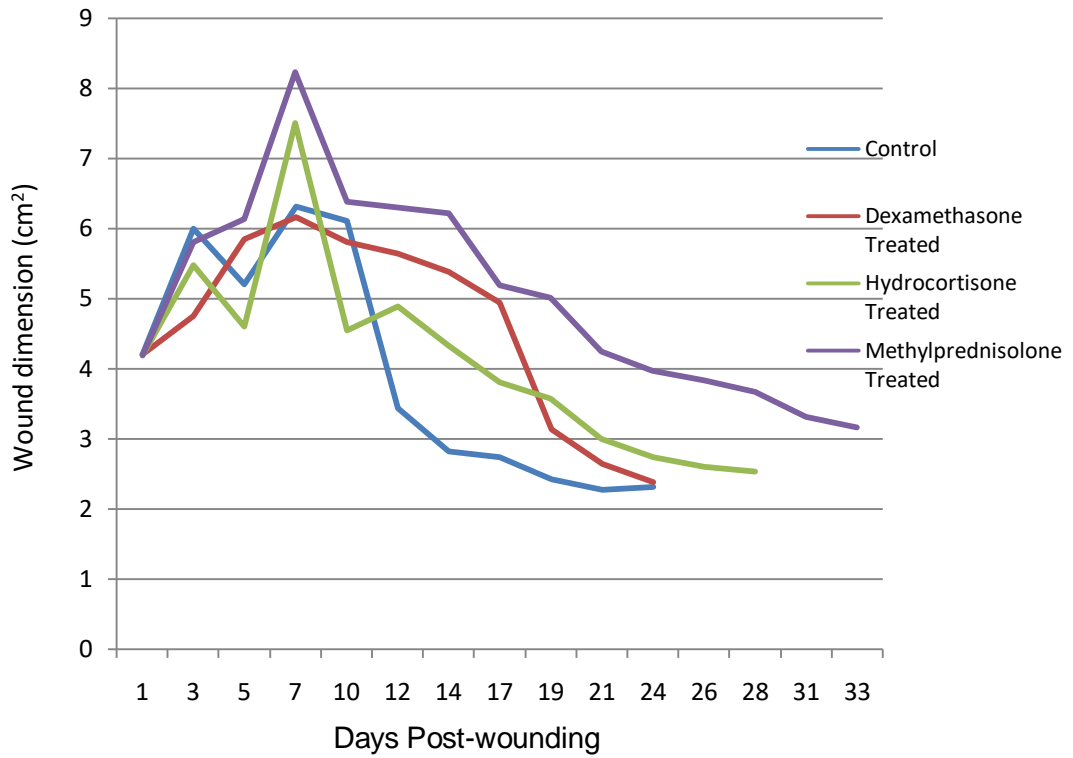


Fig.4.1.2 Mean incisional wound area in centimeter square (p-value =0.125)

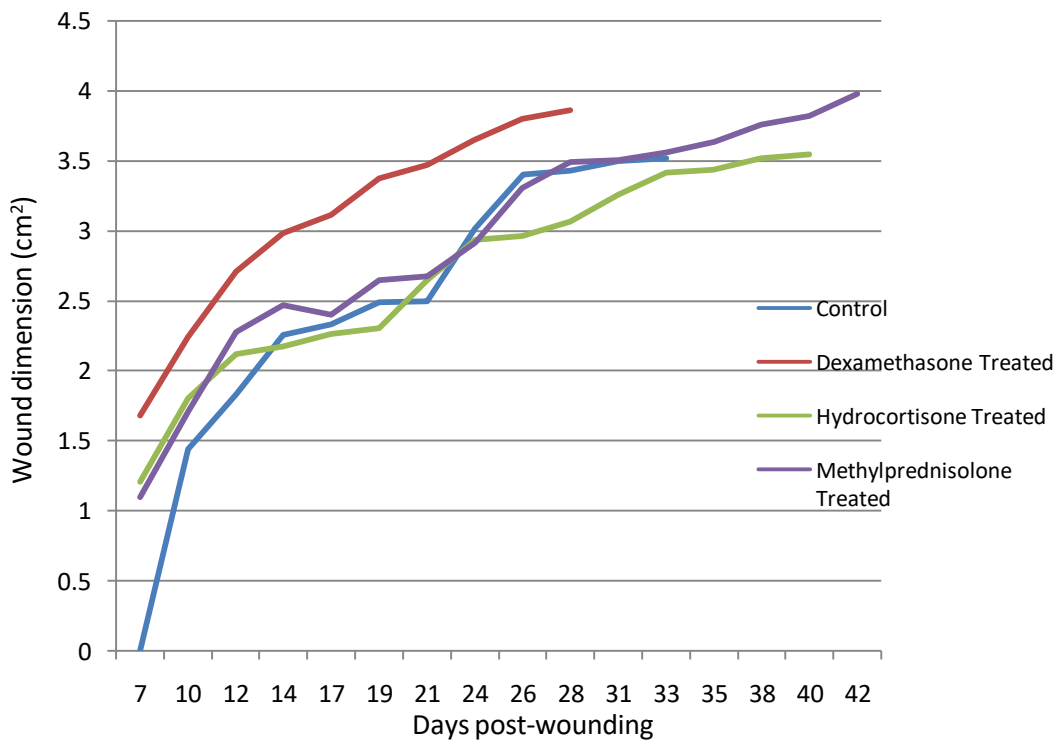


Fig. 4.1.3 Mean excisional wound epithelisation area (p-value =0.067)

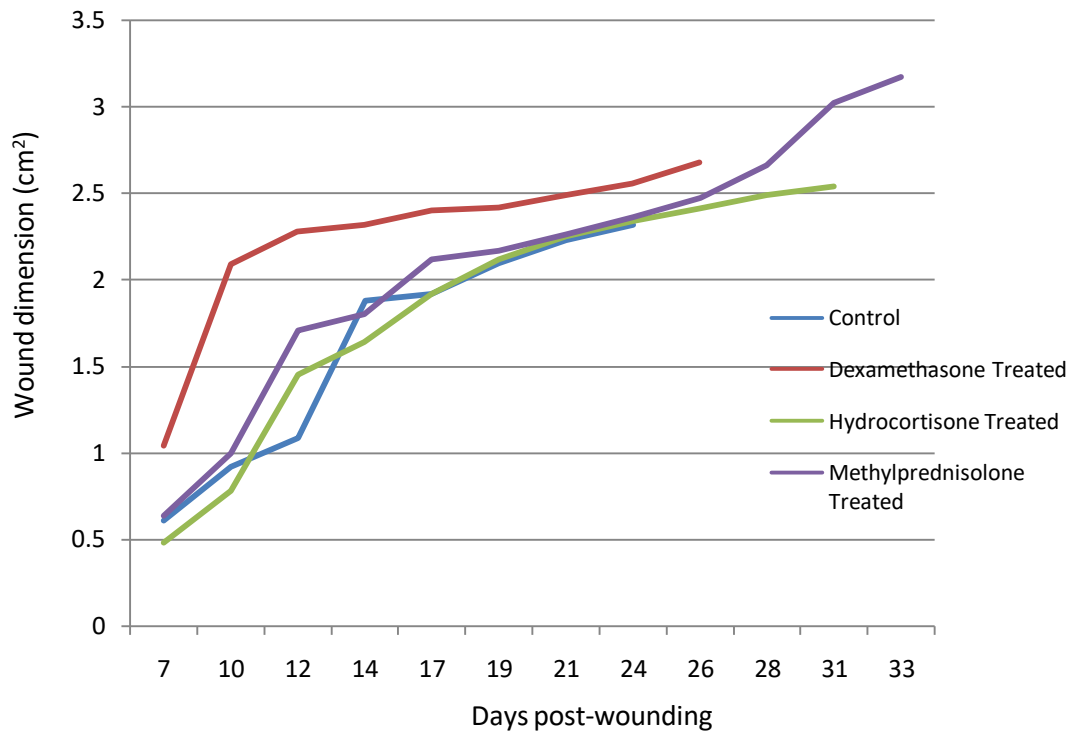


Fig. 4.1.4 Mean incisional wound epithelisation area (p-value =0.052)

4.2 Haemograms

Blood parameters considered for each of the samples were detailed as presented in Fig. 4.2.1-4.2.8 and Appendix 5. From the blood parameters, there were minor fluctuations in packed cell volume (PCV), lymphocytes and neutrophils. Subsequently, total protein and leucocyte counts showed regular fluctuations. Eosinophils appeared more in the hydrocortisone-treated group followed by the control and the dexamethasone-treated group. Methylprednisolone-treated group had the lowest number of eosinophils count. No monocytes were seen. Band cells appeared to be different statistically between groups though they were within the normal range.

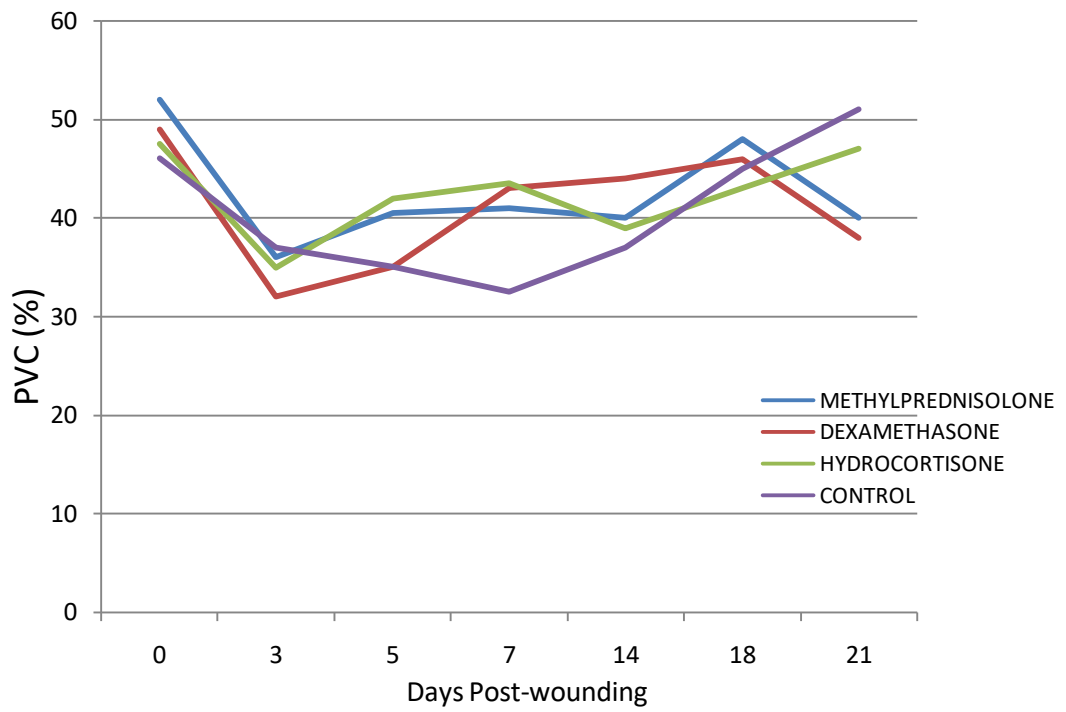


Fig. 4.2.1 Packed Cell Volume in experimental animals (p-value =0.854)

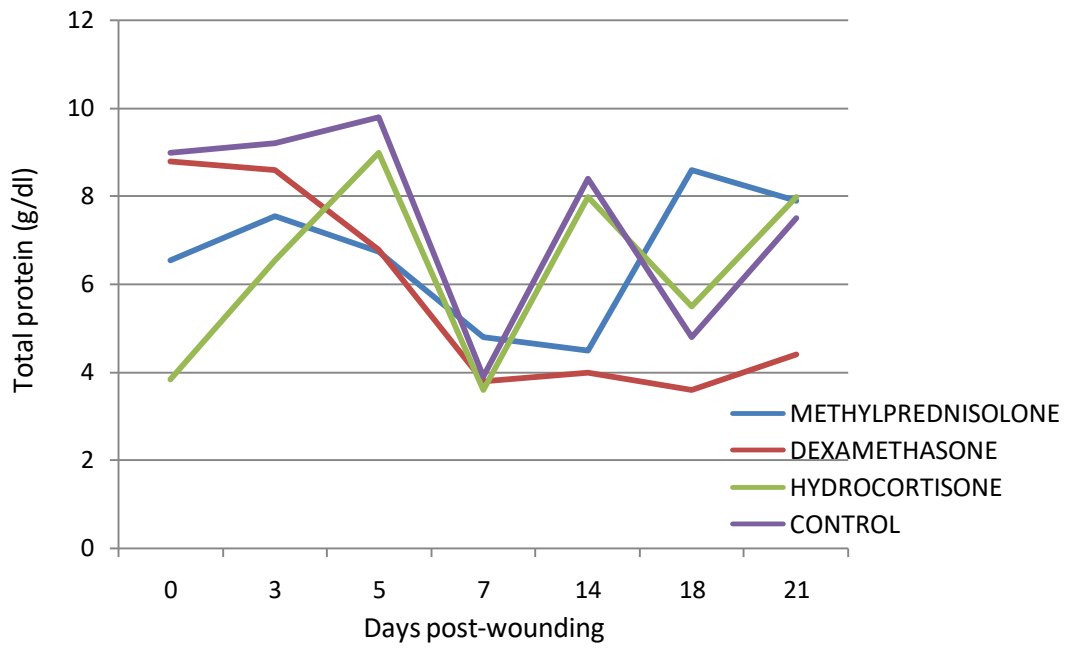


Fig. 4.2.2 Plasma protein in experimental animals (p-value =0.505)

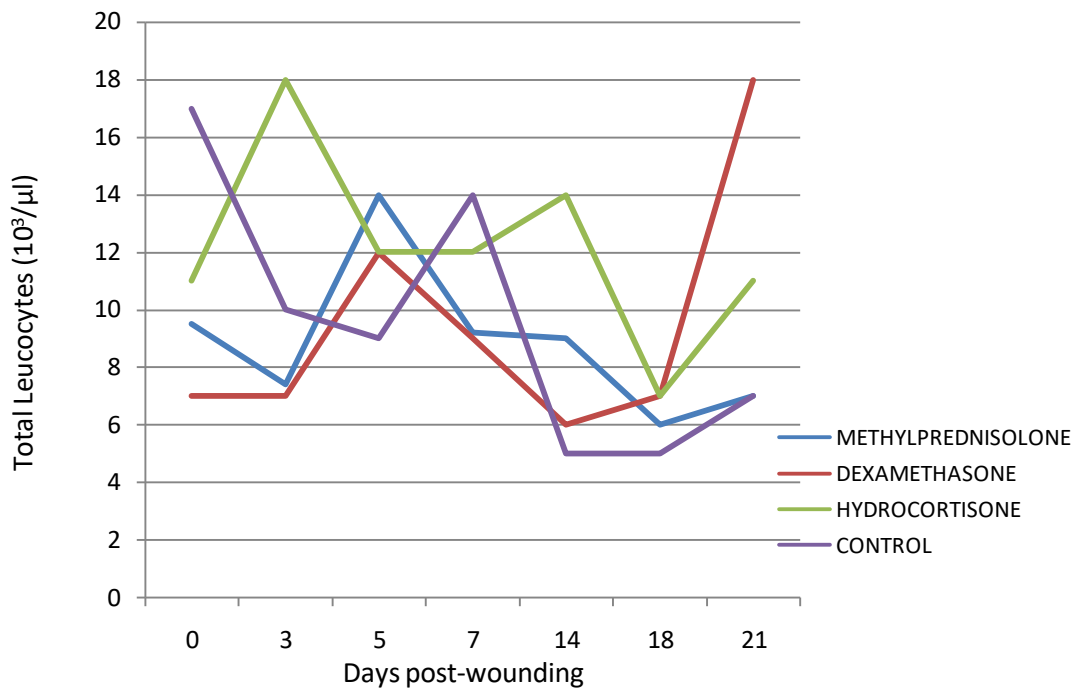


Fig. 4.2.3. Total leucocytes in experimental animals (p-value =0.447)

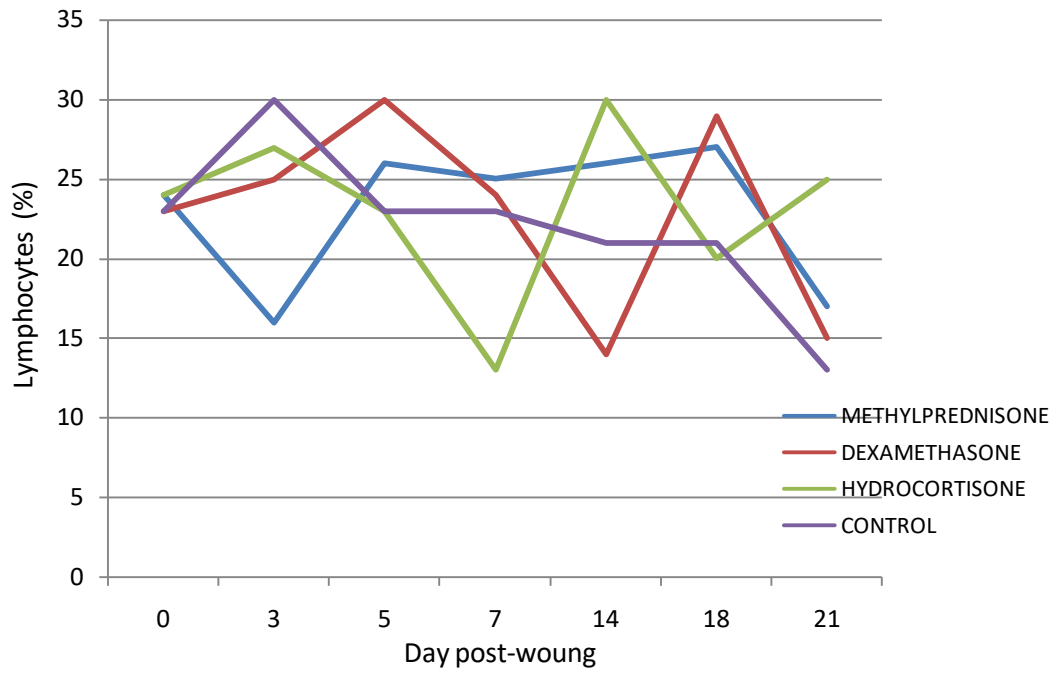


Fig.4.2.4 Lymphocytes in experimental animals (p-Value =0.234)

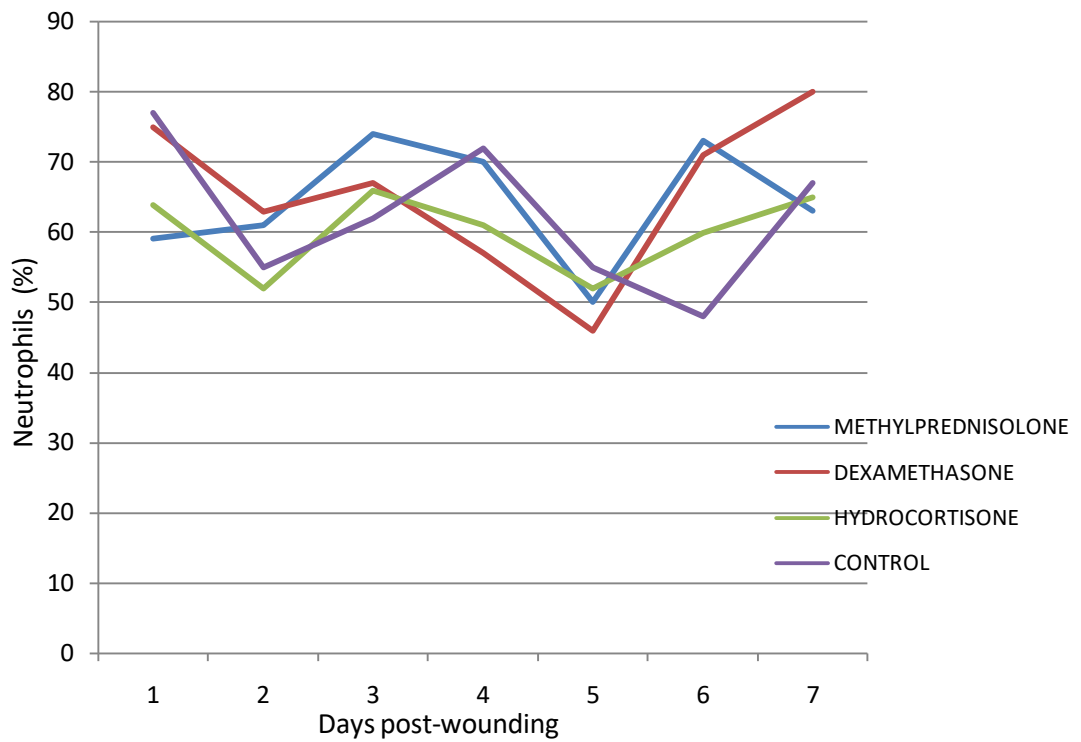


Fig.4.2.5. Neutrophils in experimental animals (p-Value =0.702)

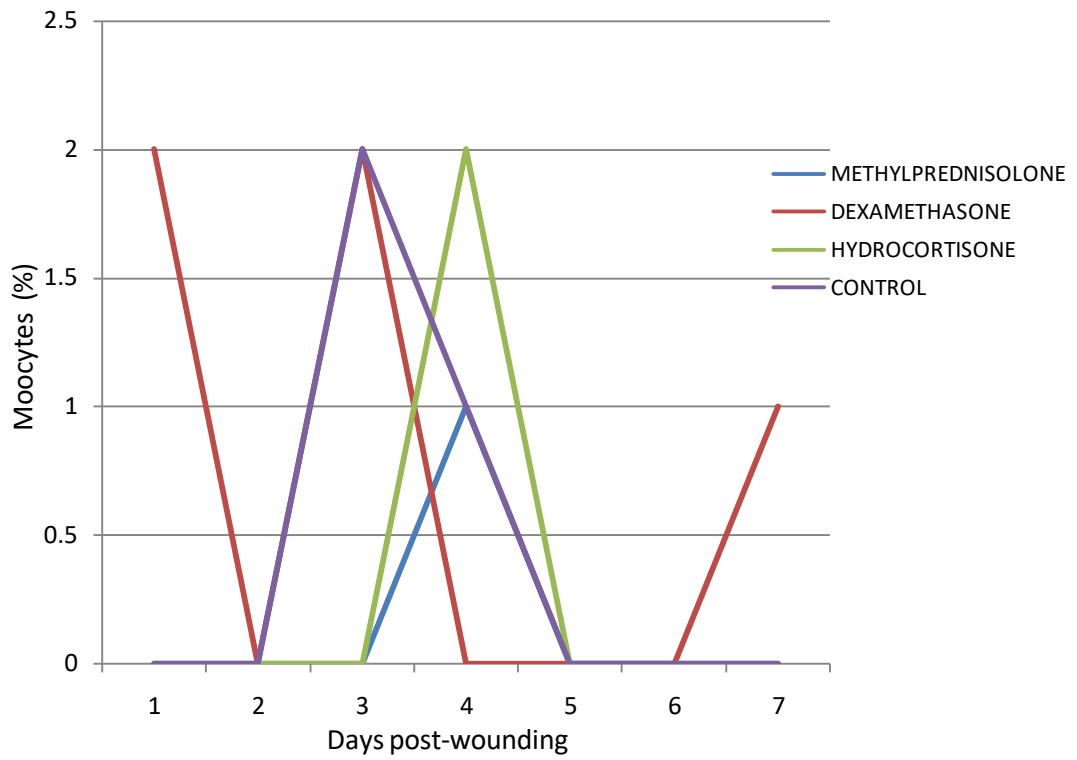


Fig. 4.26. Monocyte in experimental animals (p- Value = 0.624)

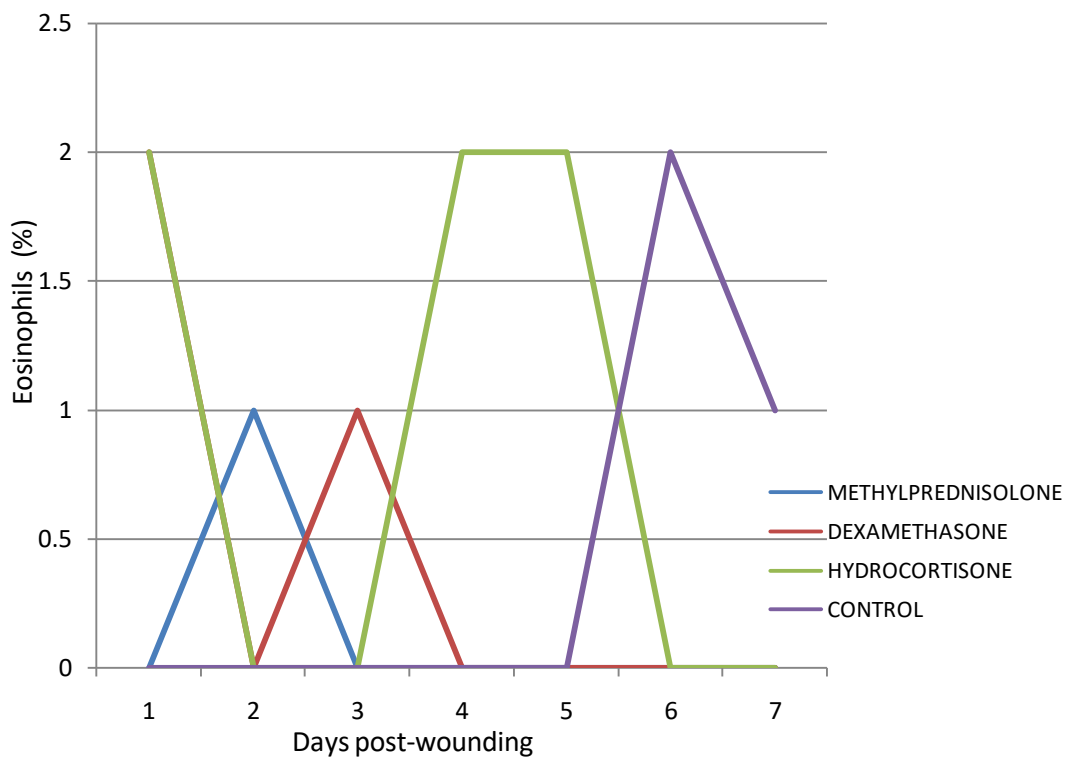


Fig.4.2.7 Eosinophils in experimental animals (p-Value =0.427)

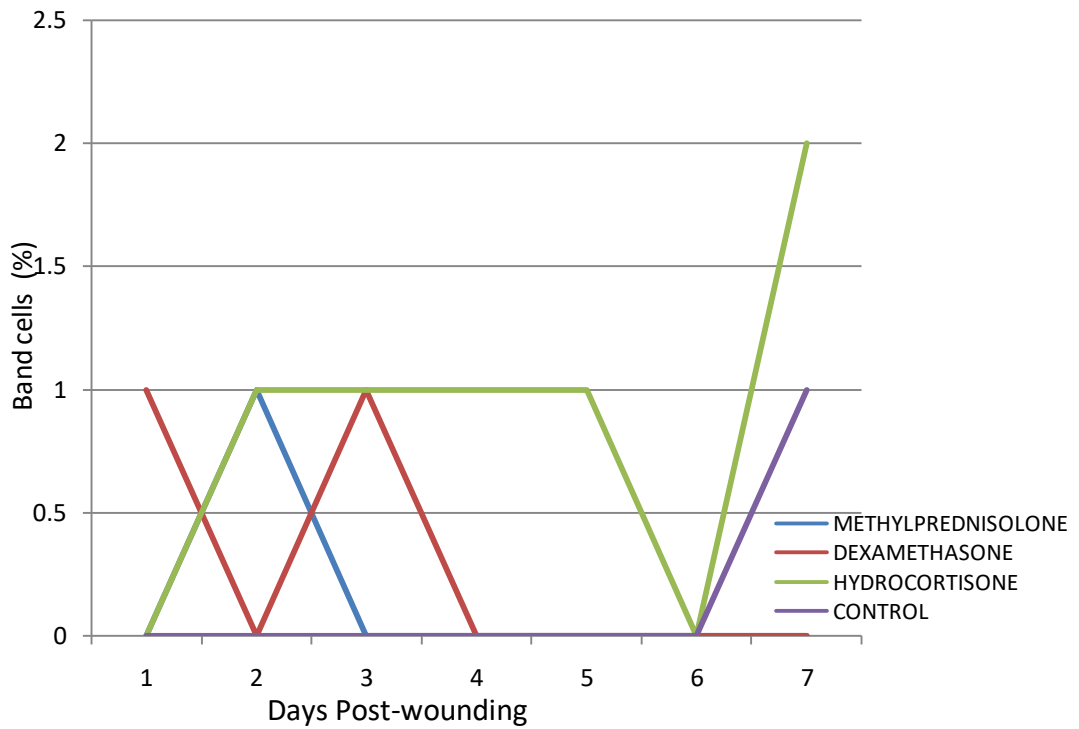


Fig. 4.2.8 Band cell in experimental animals (p-value =0.040)

4.3 Wound Healing

4.3.1 Wound suppuration

Plates IV-VII and plates XX-XXIII show the wound progression from day three post wounding, for both the incisional and excisional wounds respectively. Charmil® gel stained the healing wounds. The stain continued to day ten post wounding. Pus was also seen in all the groups from day three post wounding and persisted till charmil® gel was unable to stain the tissue again and when granulation tissue was fully formed.

4.3.2 Granulation tissue formation

Blood vessels (angiogenesis) at the wound edges were seen on day three post wounding in all the groups, on day three post-wounding. From plates XX-XXIII, one can see that the control and the methylprednisolone treated groups have a greater wound depth than the dexamethasone and hydrocortisone treated groups. In plates XXIV-XXVII, granulation bed was observed to be moderate, almost fully formed with its bright colouration and the charmil® gel stain seriously reduced. In plates XXVIII-XXXI, the bright colouration of the granulation tissue appeared to be at its peak in all the groups, however, pus was noticed in the methylprednisolone treated group. In plates VIII-XI all the groups were not stained by charmil® gel.

4.3.3 Wound contraction

The wound dimensions from the insult of the wound increases steadily to peak (10.34 cm²) at day ten in the control, 11.97 cm² day twelve, 12.21 cm² and 13.87 cm² day seventeen post-wounding in the dexamethasone, hydrocortisone and methylprednisolone treated groups respectively. Maximum contraction was recorded in this study immediately after complete epithelialisation. This occurred at day 33 (3.52 cm²) in the control, day 30 (3.86 cm²) in the dexamethasone group, day 40 (3.55 cm²) and day 42 (3.98 cm²) in the hydrocortisone and methylprednisolone group respectively as shown in tables 2 and 3. These differences which were attributed to wound contraction, however, were not statistically significant.

Table 4.1. Excisional wound contraction

Gp	Initial Wound	Final Wound	Percentage contraction (%)
----	---------------	-------------	----------------------------

	Size (cm)	Size (cm)	
Control	6	3.52	41
Hydrocortisone	6	3.86	59
Dexamethasone	6	3.55	36
Methylprednisolone	6	3.98	33

Table 4.2 Incisional wound contraction

Gp	Initial Wound Size (cm)	Final Wound Size (cm)	Percentage contraction (%)
Control	4.2	2.32	45
Hydrocortisone	4.2	2.39	43
Dexamethasone	4.2	2.54	39
Methylprednisolone	4.2	3.17	24

4.3.4 Wound epithelialisation

Complete epithelialisation was said to have been achieved when epithelium covers the entire wound surface. This was seen on day 30 post-wounding for the control and dexamethasone-treated groups and days 32 and 35 for the hydrocortisone and methylprednisolone group respectively all for the excisional wounds. Epithelialisation was clearly represented in plates XII-XV and plates XXVIII-XXXI.

The incisional wounds were presented in plates IV-XIX as wounds progressively heal, while plates XX-XXV show pictures of excisional wounds reflecting the healing progression.

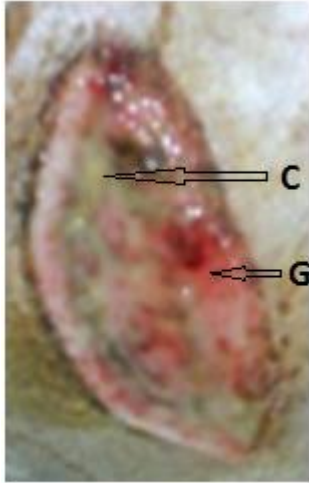


Plate IV. Control group day 3.

Note:- Charmil (C),
Granulation tissue (G).

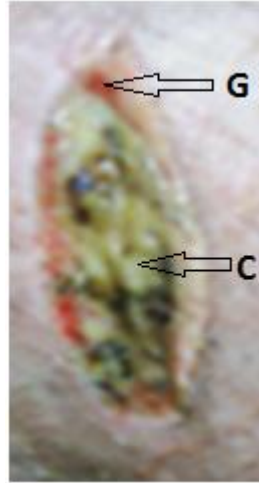


Plate V. Hydrocortisone group

day 3. Note:- Granulation tissue (G),
charmil (C).

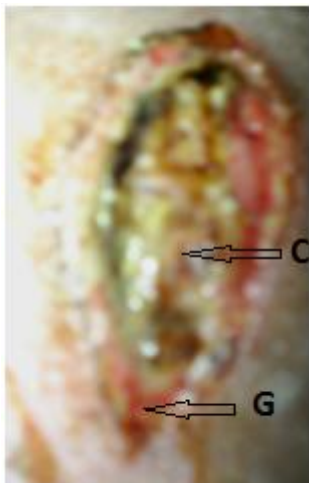


Plate VI. Dexamethasone

group day 3. Note:- Charmil (C),
Granulation tissue (G).



Plate VII. Methylprednisolone

group day 3. Note:- Granulation
tissue (G), charmil (C).

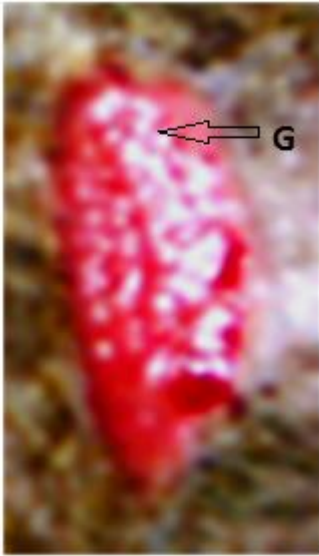


Plate VIII. Control group day 7.

Note:- Charmil (C),
Granulation tissue (G).

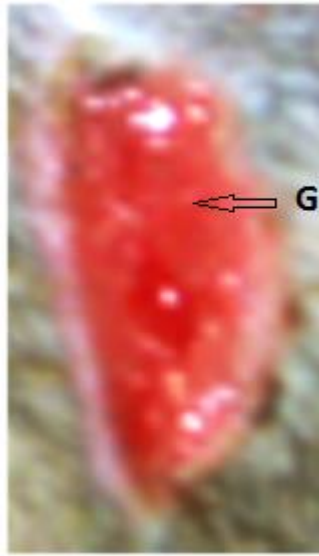


Plate IX. Hydrocortisone

Sgroup day 7. Note:- Granulation
tissue (G), charmil (C).

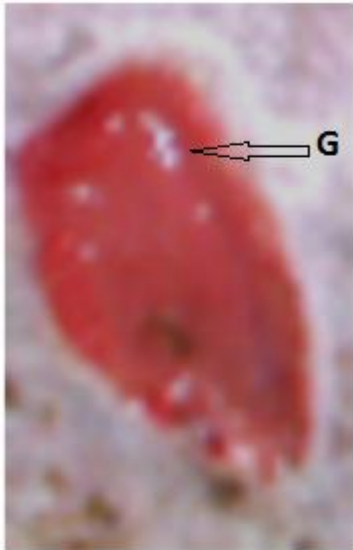


Plate X. Dexamethasone group
day 7. Note:- Charmil (C),
granulation tissue (G).



Plate XI.
Methylprednisolone group day 7
Note:- Granulation tissue (G),
charmil (C).

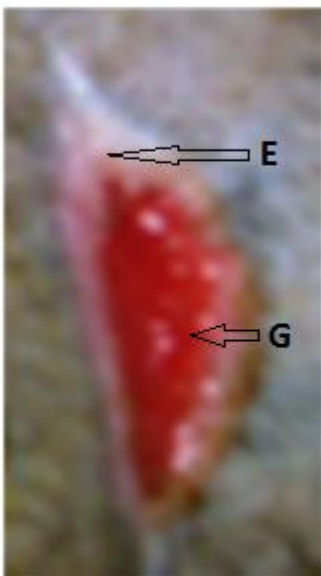


Plate XII. Control group
day 14. Note:- Epithelisation (E),
Granulation tissue (G).

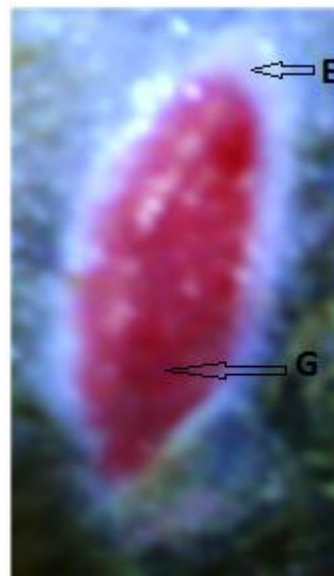


Plate XIII. Hydrocortisone group
day 14. Note:- Granulation tissue (G),
Epithelisation (E).



Plate XIV.

Dexamethasone group day 14.

Note:- Epithelisation (E),
Granulation tissue (G).

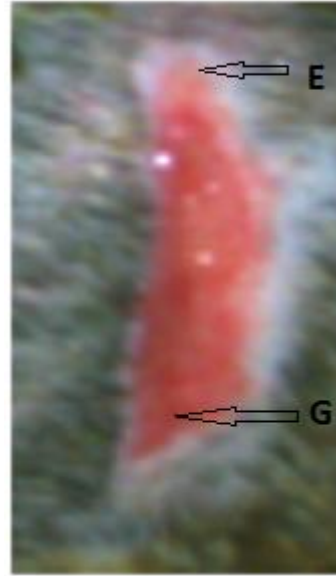


Plate XV

Methylprednisolone group day 7.

Note:- Granulation tissue (G),
Epithelisation (E).

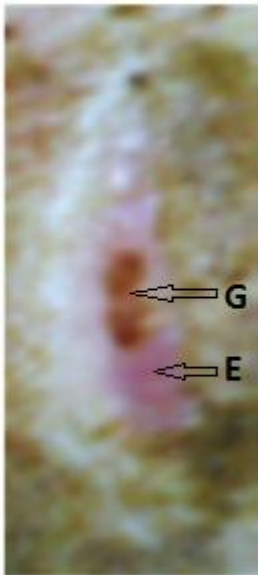


Plate XVI. Control group day 21.

Note:- Epithelisation (E),
Granulation tissue (G).

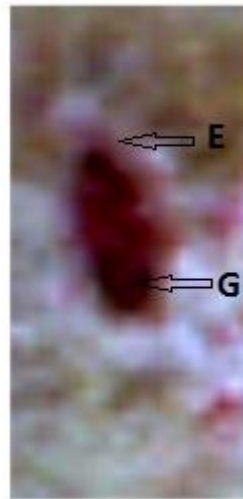


Plate XVII. Hydrocortisone

group day 21. Note:-
Granulation tissue (G), Epithelisation (E).

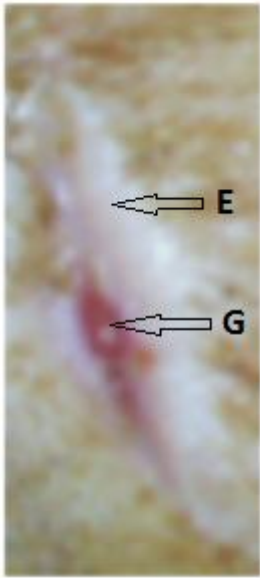


Plate XVIII. Dexamethasone group
day 21. Note:- Epithelisation (E),
Granulation tissue (G).



Plate XIX.
Methylprednisolone group day 21
Note:- Granulation tissue (G)
Epithelisation (E).

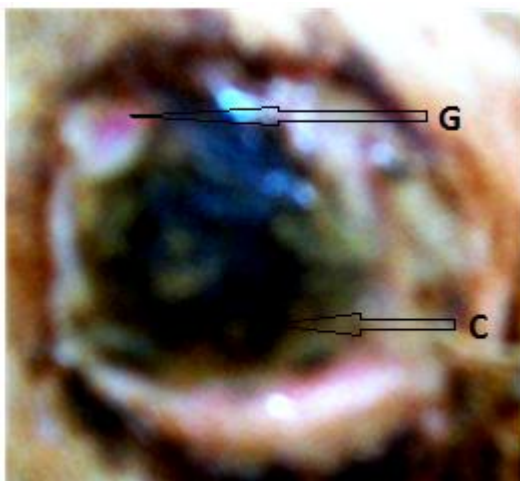


Plate XX. Control group day 3.
Granulation tissue (G).

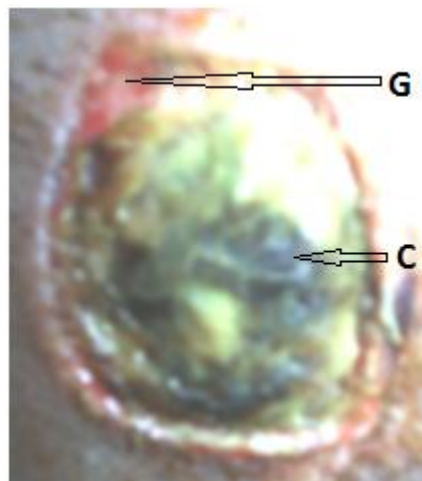


Plate XXI. Hydrocortisone group day 3. Note:-
Epithelisation (E),
Granulation tissue (G)

Epithelisation (E).

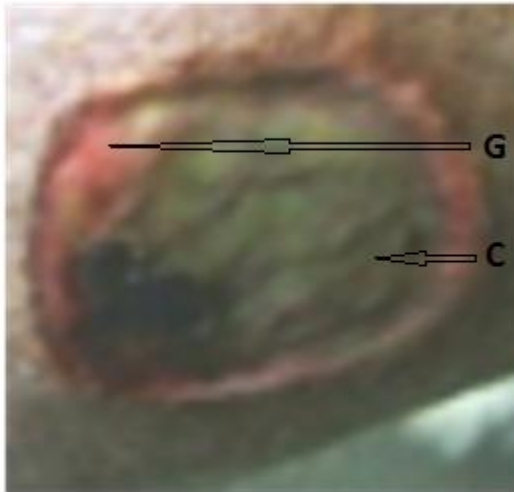


Plate XXII. Dexamethasone group
day 3. Note:- Epithelisation (E),
Granulation tissue (G).

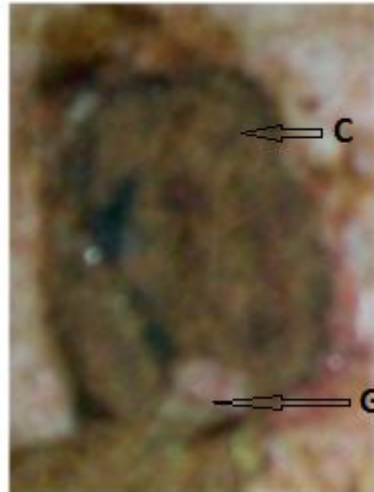


Plate XXIII.
Methylprednisolone group day 3.
Note:-Granulation tissue (G),
Epithelisation(E).

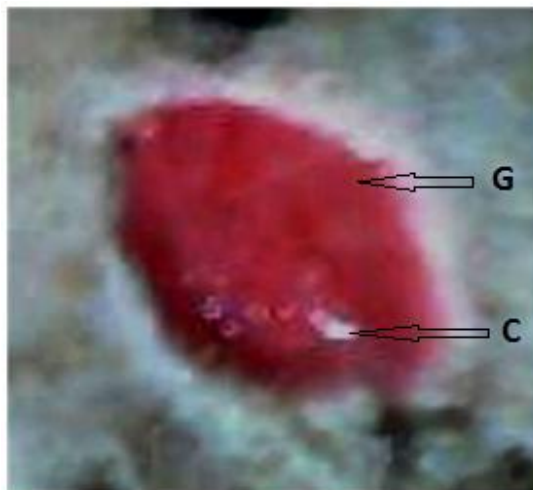


Plate XXIV. Control group day 7
Note:- Epithelisation (E),
Granulation tissue (G).

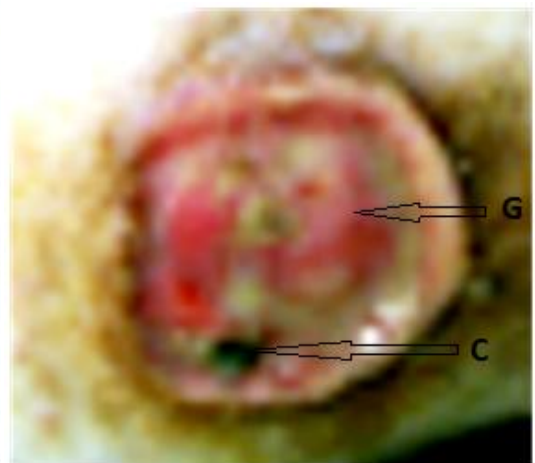


Plate XXV. Hydrocortisone
group day 7. Note:- Granulation
tissue (G), Epithelisation (E).

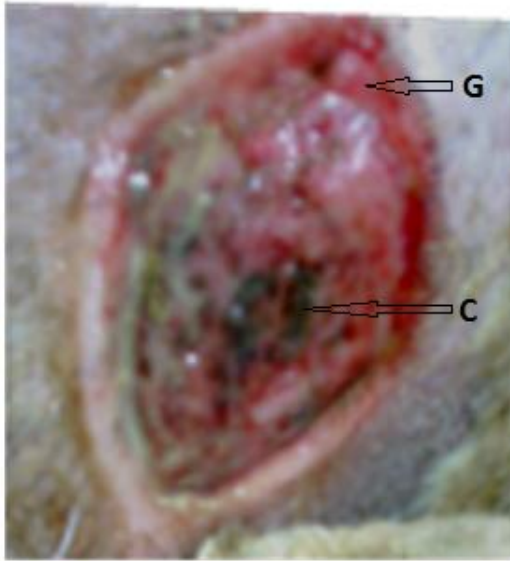


Plate XXVI. Dexamethasone group
day 7. Note:- Epithelisation (E),
Granulation tissue (G).

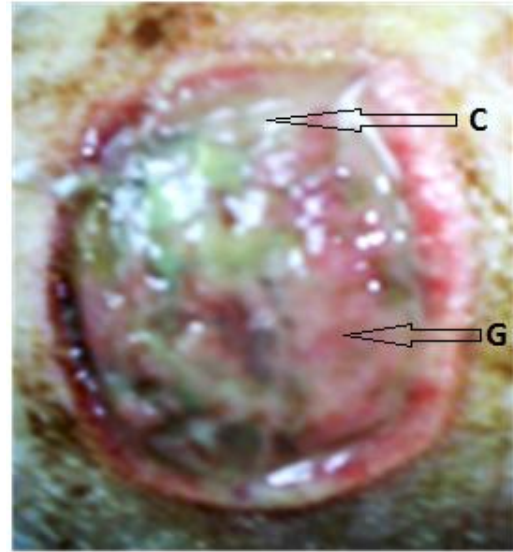


Plate XXVII.
Methylprednisolone group day 7.
Note:-Granulation tissue (G),
Epithelisation (E).

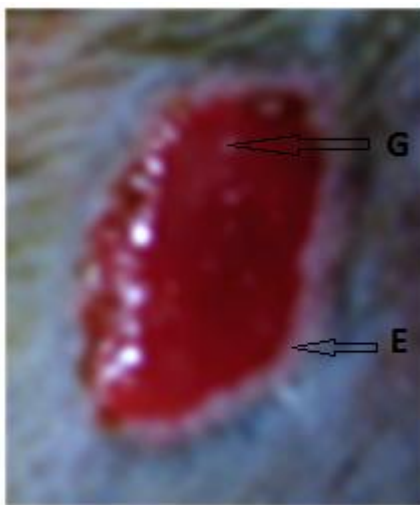


Plate XXVIII. Control group

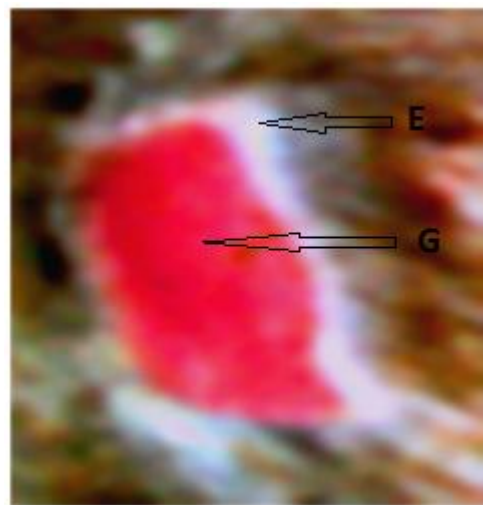


Plate XXIX. Hydrocortisone

day 14 Note:- Epithelisation (E), group day 14. Note:- Granulation tissue (G), Epithelisation (E).

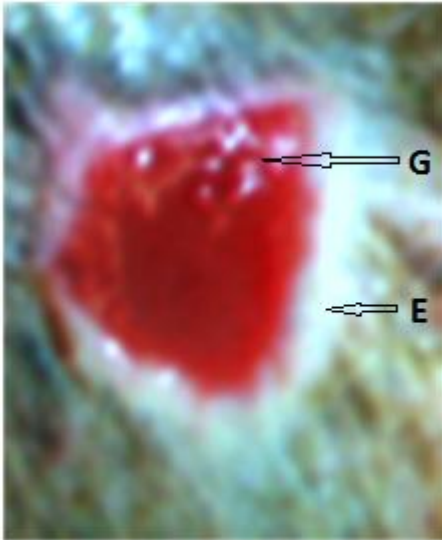


Plate XXX. Dexamethasone group day 14. Note:- Epithelisation (E), Granulation tissue (G).

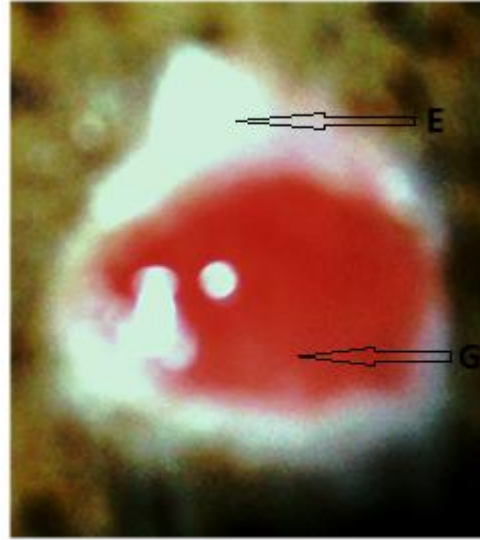


Plate XXXI. Methylprednisolone group day 14. Note:- Granulation tissue (G), Epithelisation (E).

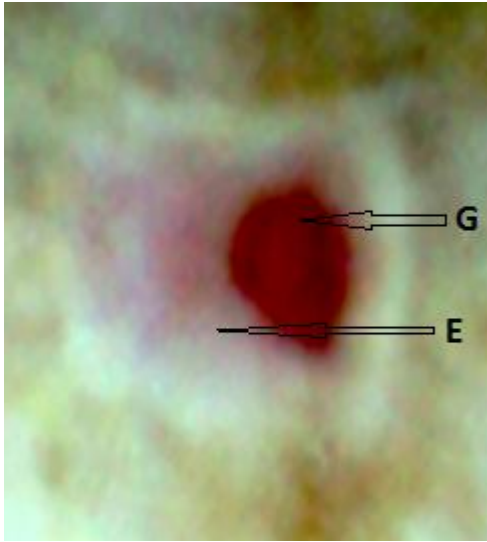


Plate XXXII. Control group
day 21. Note:- Epithelisation (E),
Granulation tissue (G).

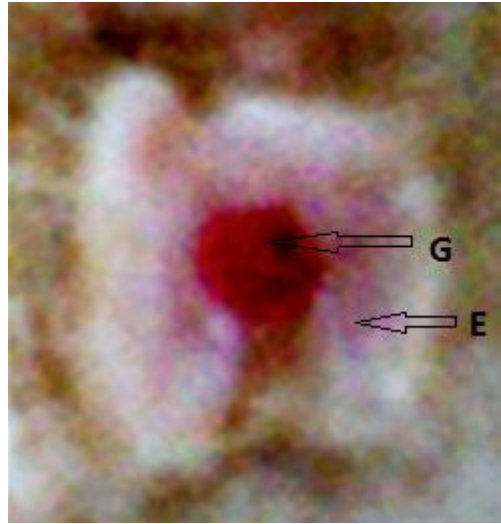


Plate XXXIII.
Hydrocortisone group on day
21. Note:- Granulation tissue (G),
Epithelisation (E).

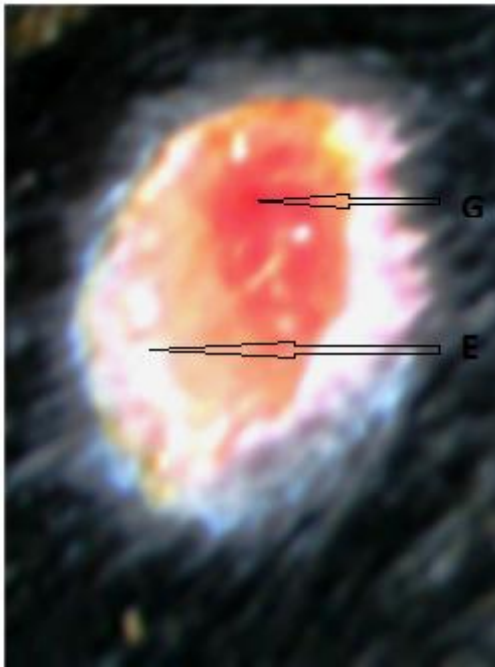


Plate XXXIV. Dexamethasone
group day 21. Note:- Epithelisation (E),
Granulation tissue (G).

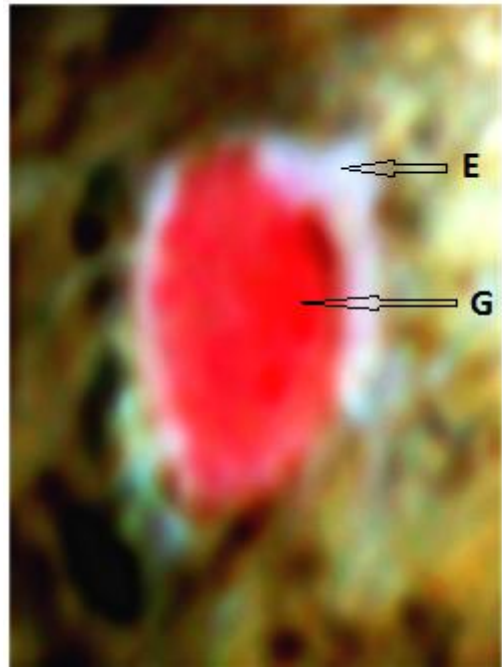


Plate XXXV.
Methylprednisolone group
day 21. Note:-

Granulation tissue (G), Epithelisation (E).

4.4.1. Histological progression of wound healing

Day 3 post-wounding: Numerous inflammatory cells were seen in the control (Plate XXXVI), the inflammatory cells, though present in the steroid treated groups were relatively less in the hydrocortisone and dexamethasone-treated groups (Plates XXXVII and XXXVIII). The methylprednisolone-treated group (Plate XXXVIII) showed the least infiltration of inflammatory cells.

Day 7 post-wounding: Inflammatory cells were present in the granulation tissue of the control (Plate XIL), hydrocortisone and dexamethasone-treated groups (Plates XL and XLI). Relatively less inflammatory cells were observed in the methylprednisolone (Plate XLII) treated group which had more organised granulation tissue.

Day 14 post-wounding: Neovascularisation was present with slight inflammatory cellular infiltration in the control (Plate XLIII), hydrocortisone and dexamethasone-treated groups (Plates XLIV and XLV). The dexamethasone-treated group also showed numerous blood vessels. The methylprednisolone group (Plate XLVI) in addition showed commencement of epithelialisation.

Day 21 post-wounding: The control and the hydrocortisone-treated group (Plates XLVII and XLVIII) showed well formed granulation tissue, of all the groups neovascularisation is more pronounced in the dexamethasone-treated group

Days 30-35: The scar were taken on day 30 in the control group (Plate LI) and dexamethasone group (plate LIII), day 32 in the hydrocortisone group (Plate LII), and day 35 in the methylprednisolone group (Plate LIV). Complete epithelialisation occurred in all the groups with more organised cells in the methylprednisolone group, which was also confirmed using the Verhoeff-Van Giesen stain (VVG), which is specific for collagen, cell nucleus and elastic fibres. Verhoeff-Van Giesen stain, stains collagen red, nucleus black, elastic fibres black and muscles yellow. Collagen deposit as shown in the scar tissue stained with Verhoeff-Van Giesen stain (Plate LIV - LVIII)

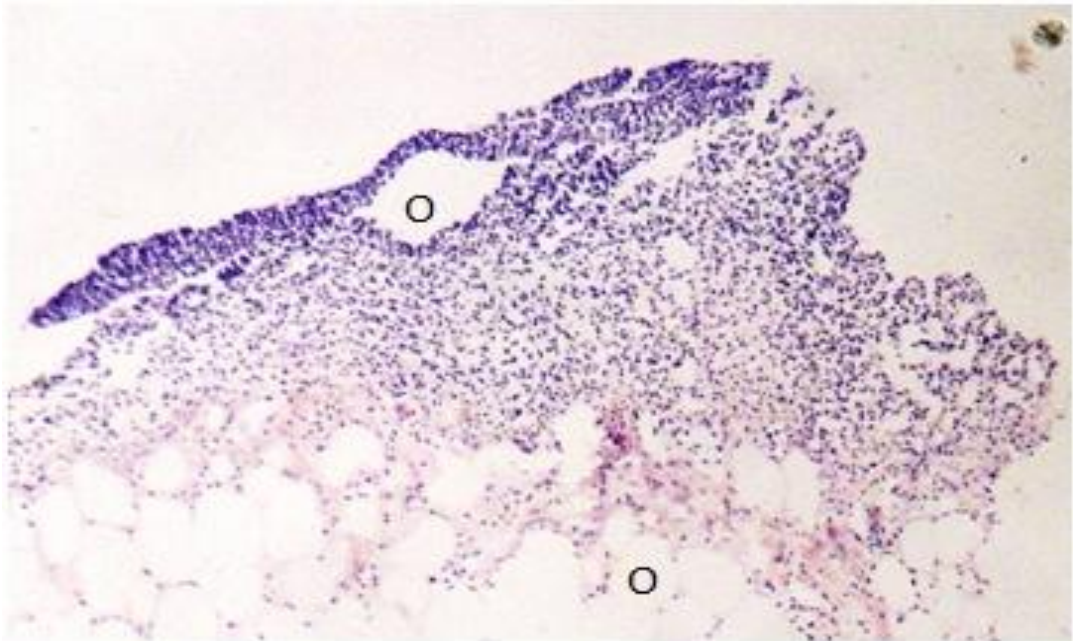


Plate XXXVI- Photomicrograph of the skin of dog in control (untreated) group taken three days post-wounding. Note :- edema (o) with diffused inflammatory cells. H & E \times 100

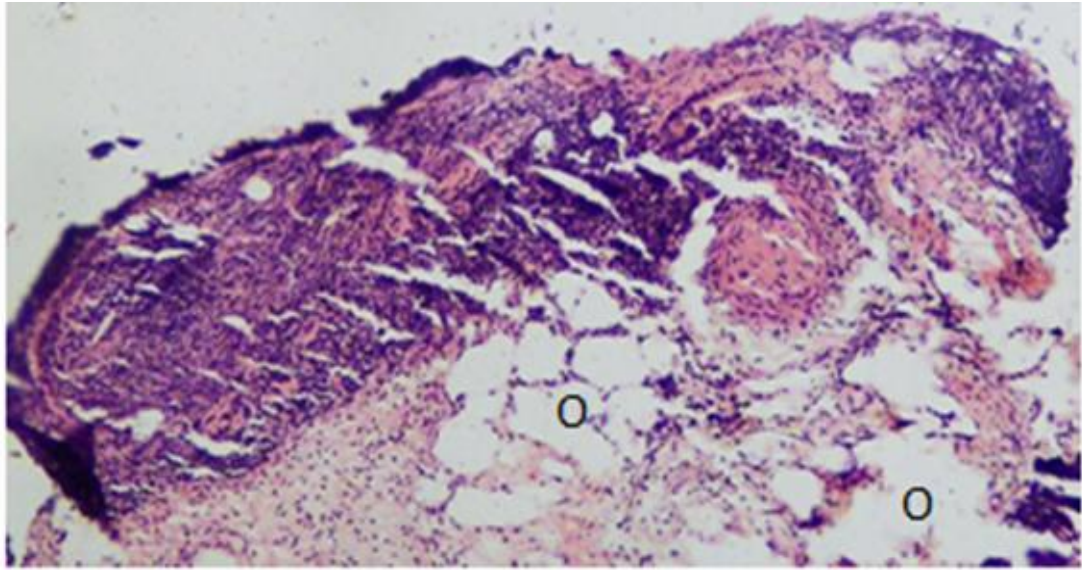


Plate XXXVII- Photomicrograph of the skin of dog in hydrocortisone treated group taken three days post-wounding. Note :- edema (o) with diffused inflammatory cells. H & E \times 100

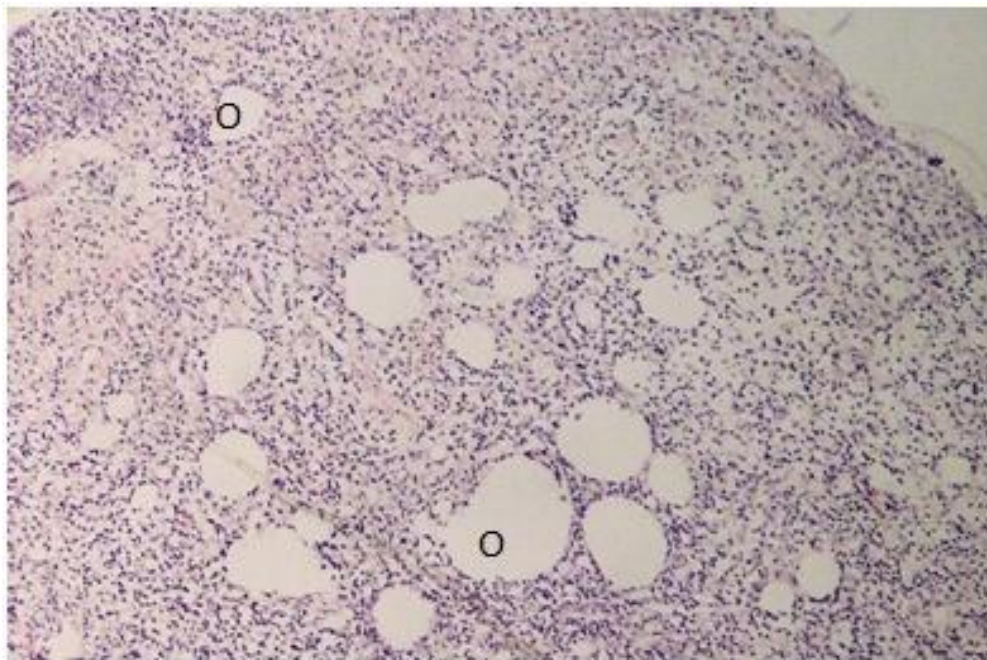


Plate XXXVIII- Photomicrograph of the skin of dog in dexamethasone treated group taken three days post-wounding. Note :- edema (o) with diffused inflammatory cells. H & E \times 100

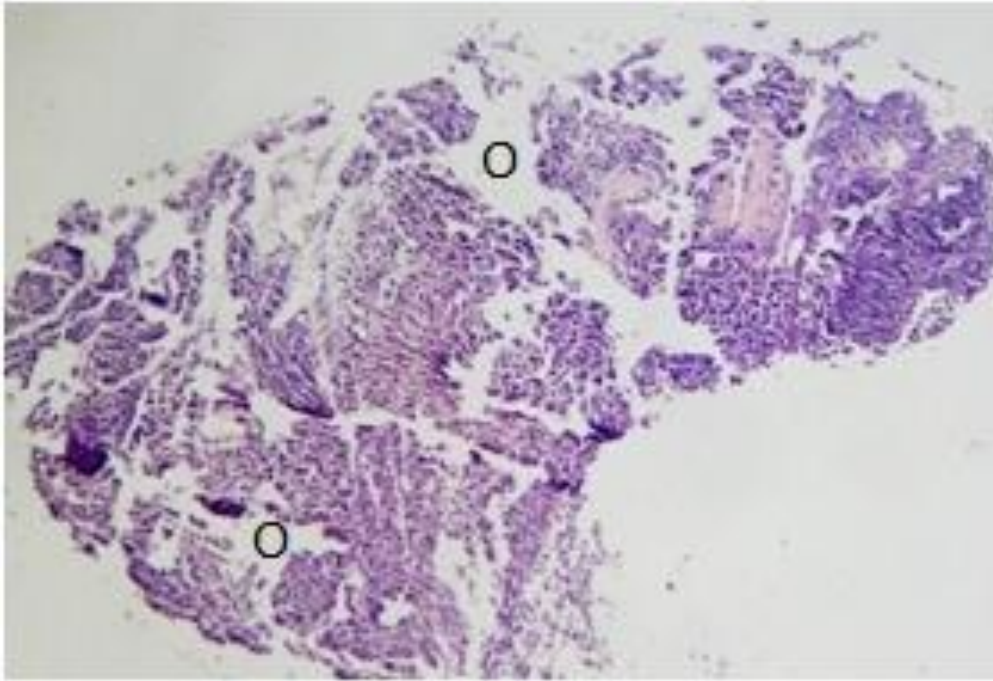


Plate XII- Photomicrograph of the skin of dog in methylprednisolone treated group taken three days post-wounding. Note :- edema (o) with diffused inflammatory cells. H & E \times 100

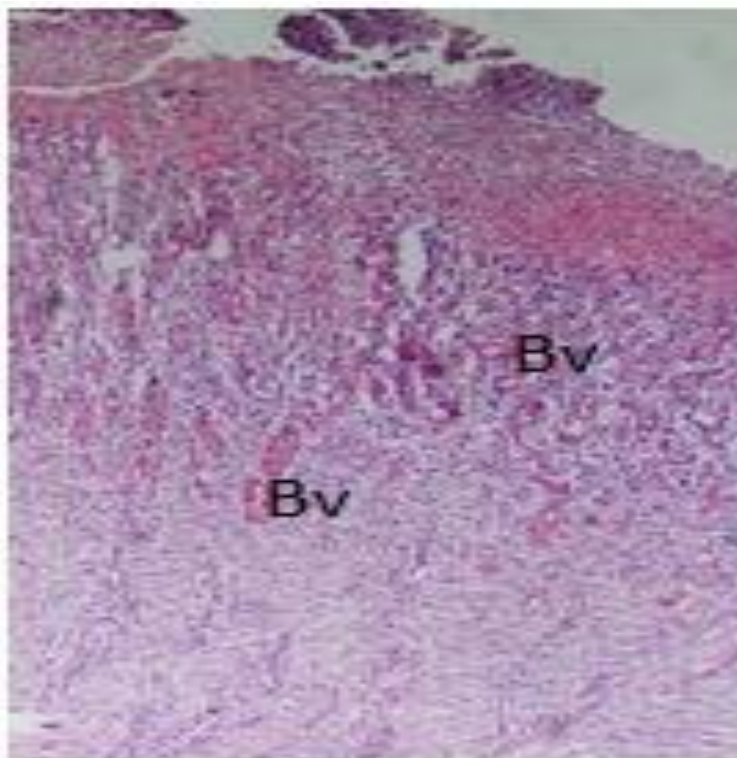


Plate XI- Photomicrograph of the skin of dog in control (untreated) group taken seven days post-wounding. Note :- Blood vessels (Bv), with mild infiltration of inflammatory cells. H & E \times 100

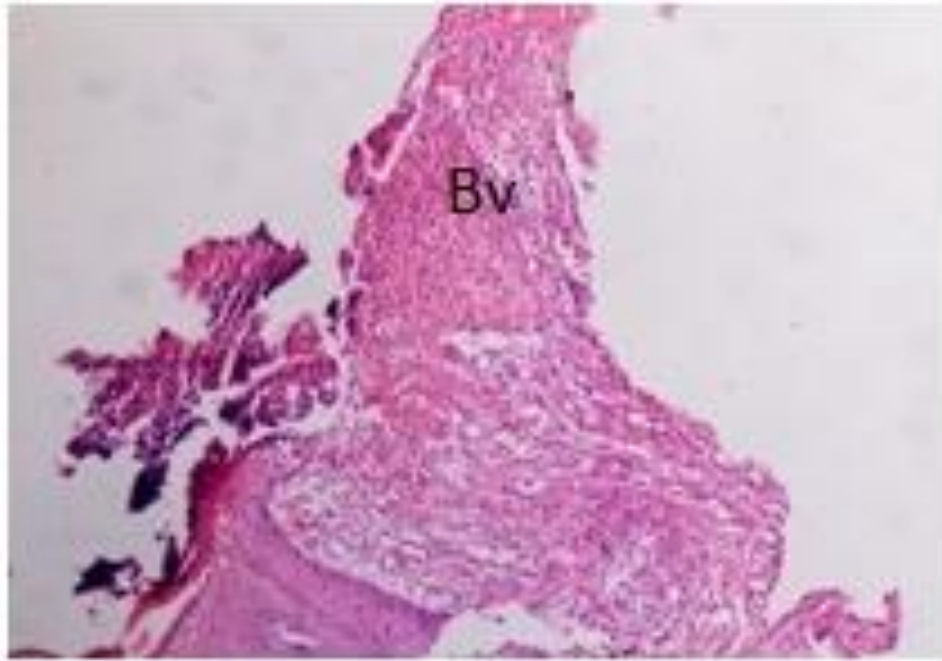


Plate XLI- Photomicrograph of the skin of dog treated with hydrocortisone taken seven days post wounding:- Blood vessels (Bv) which are scanty, with mild infiltration of inflammatory cells. H & E \times 100

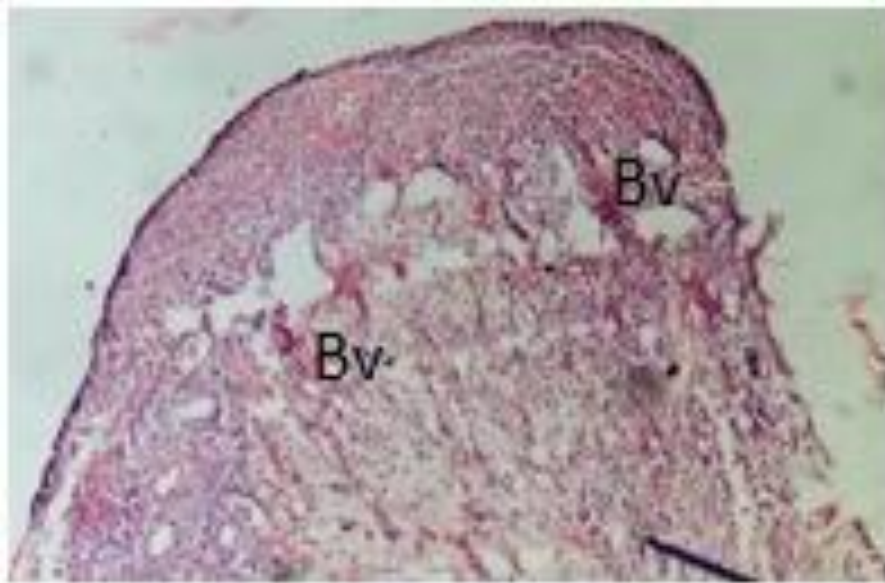


Plate XLII- Photomicrograph of the skin of dog treated with dexamethasone taken seven days post wounding:- Numerous blood vessels (Bv), with moderate infiltration of inflammatory cells. H & E \times 100

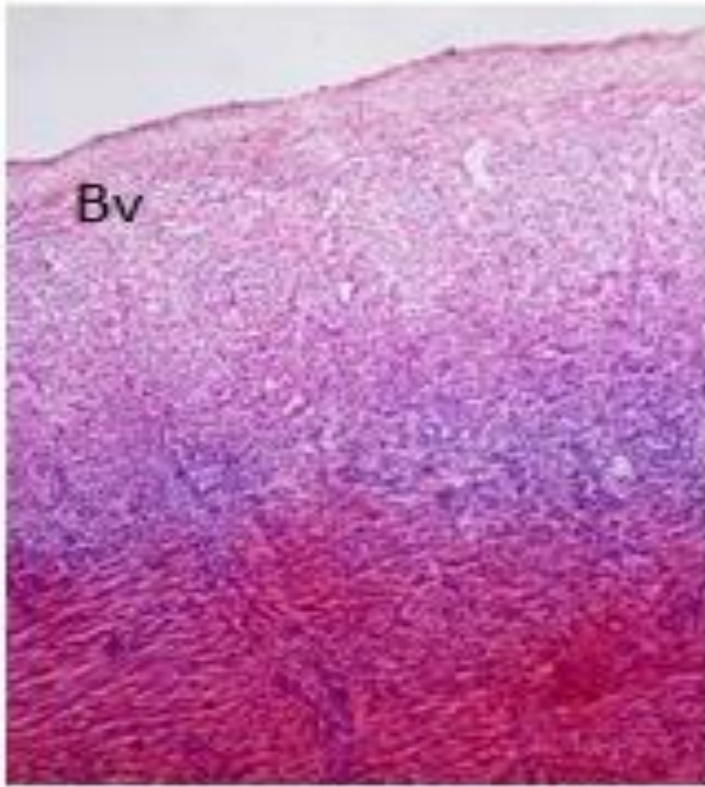


Plate XLIII- Photomicrograph of the skin of dog treated with methylprednisolone seven days post wounding:- Scanty blood vessels (Bv), with mild infiltration of inflammatory cells. H & E \times 100

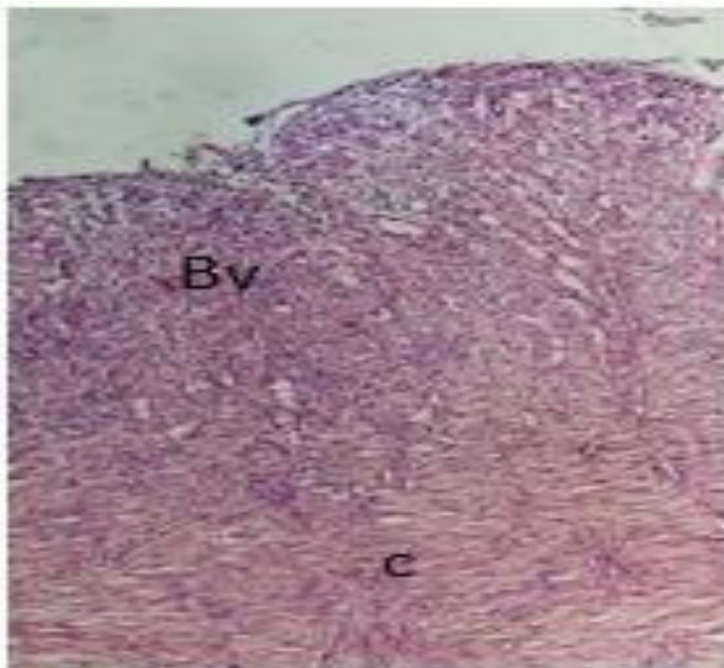


Plate XLIV- Photomicrograph of the skin of dog in control (untreated) taken fourteen days post wounding :- Collagen (c) blood vessels (Bv), with mild infiltration of inflammatory cells. H & E \times 100

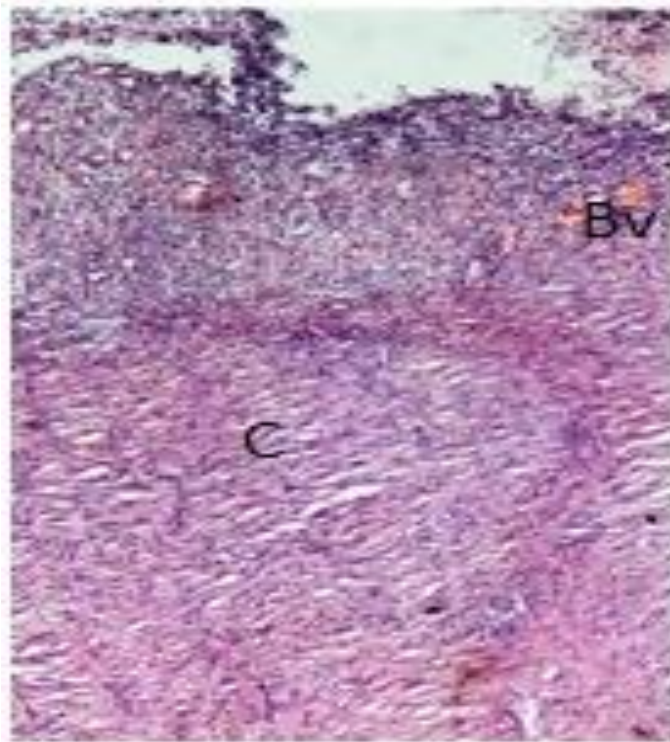


Plate XLV- Photomicrograph of the skin of dog treated with hydrocortisone taken fourteen days post wounding :- Collagen (c), Blood vessels (Bv), with mild infiltration of inflammatory cells. H & E \times 100

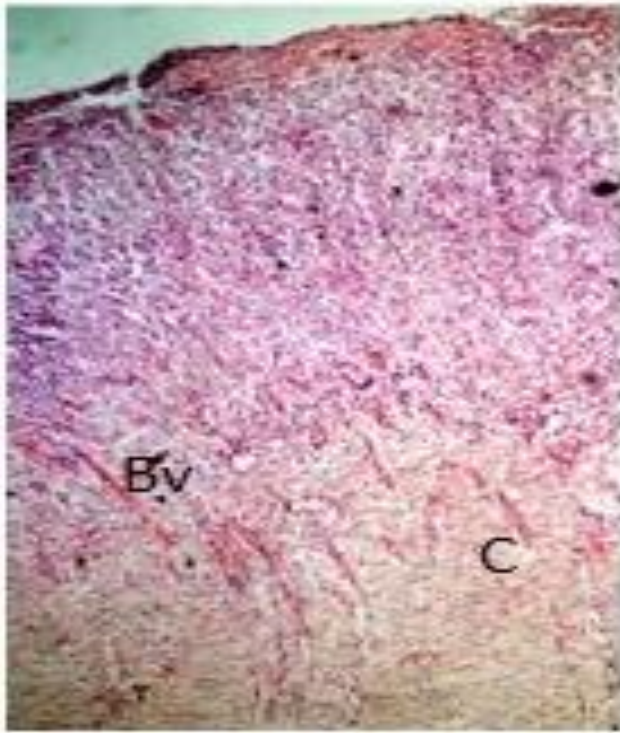


Plate XLVI- Photomicrograph of the skin of dog treated with dexamethasone taken fourteen days post wounding :- Collagen (c), with numerous blood vessel (Bv), H & E x 100.

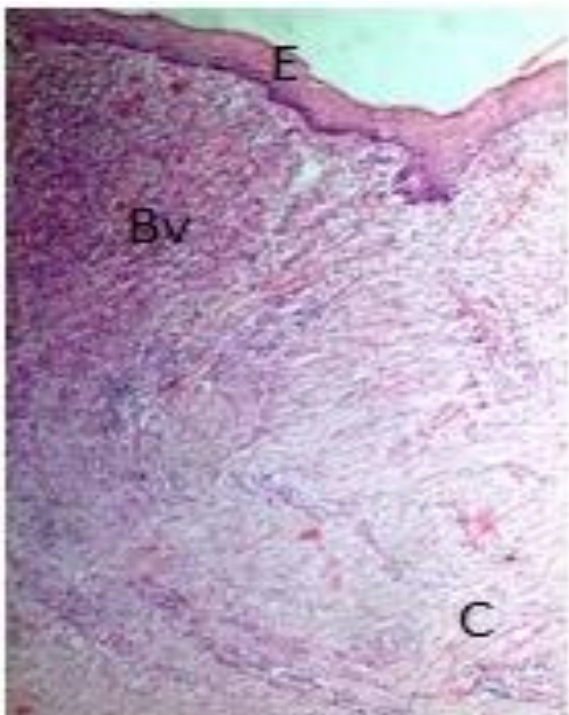


Plate XLVII- Photomicrograph of the skin of dog treated with methylprednisolone taken fourteen days post wounding :- Collagen (c), epithelium (E), blood vessel (Bv). H & E x 100.

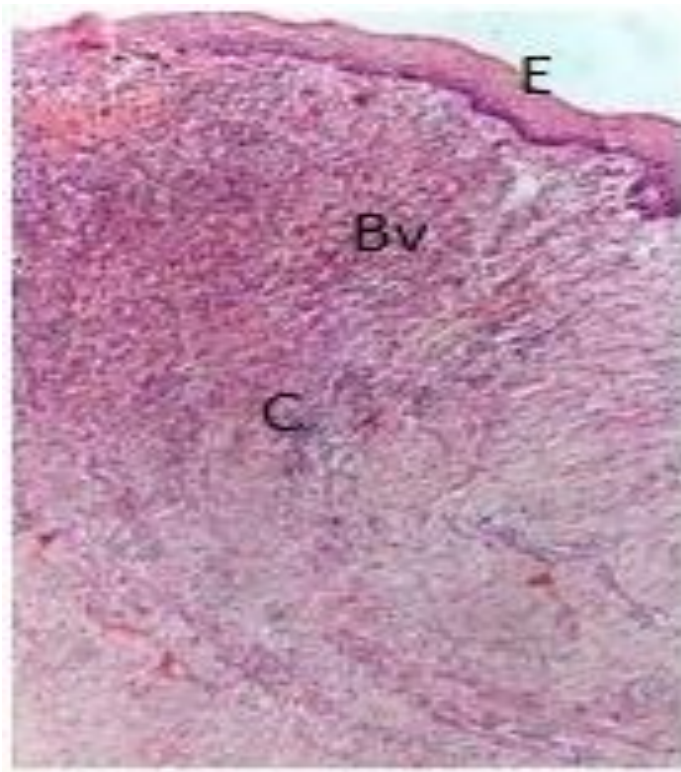


Plate XLVIII- Photomicrograph of the skin of dog in control (untreated) taken twenty one days post wounding :- Collagen (c), epithelium (E), blood vessel (Bv), H & E x 100.

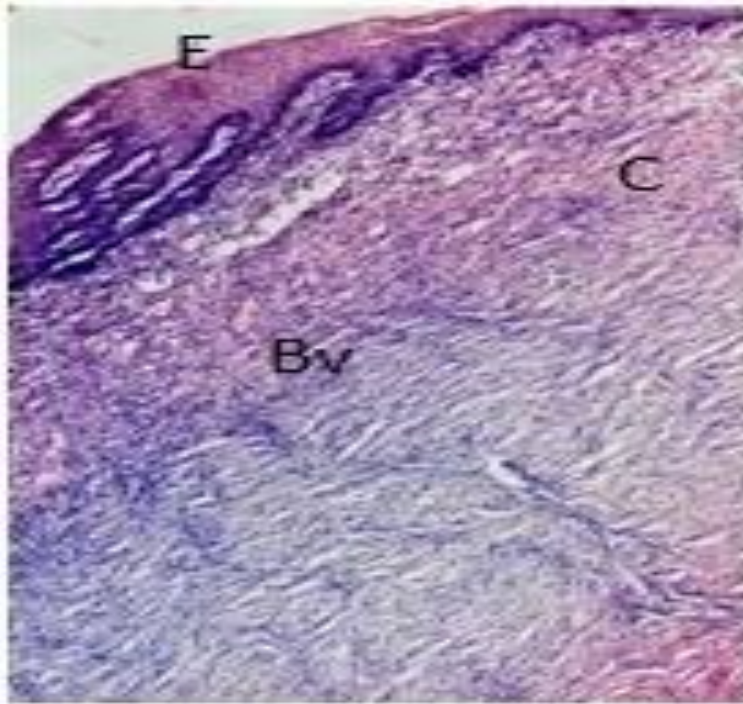


Plate II- Photomicrograph of the skin of dog treated with hydrocortisone taken twenty one days post wounding :- Collagen (c), epithelium (E), with scanty blood vessel (Bv), H & E x 100.

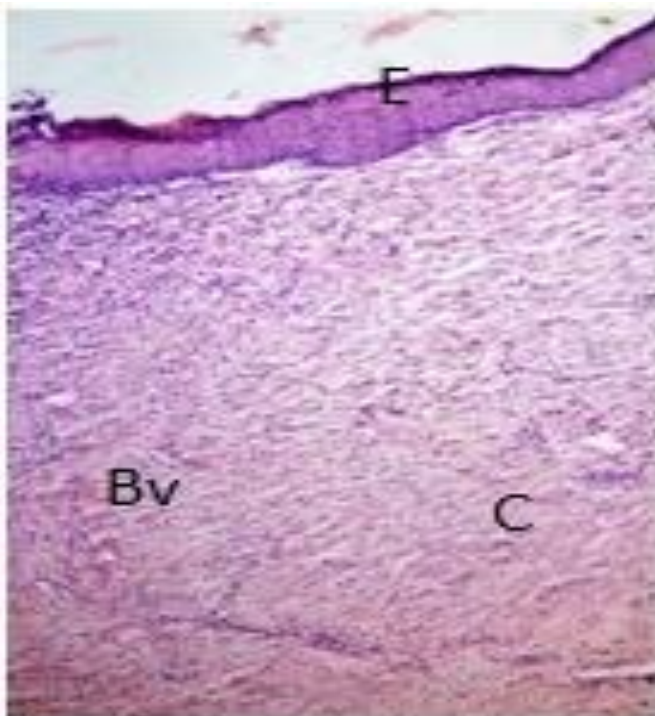


Plate L- Photomicrograph of the skin of dog treated with dexamethasone taken twenty one days post wounding :- Collagen (c), epithelium (E), scanty blood vessel (Bv), H & E x 100.

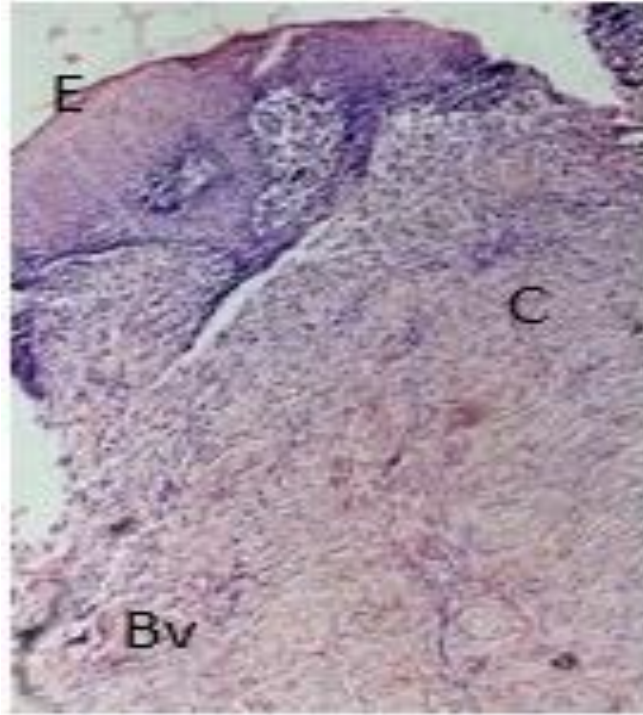


Plate LI- Photomicrograph of the skin of dog treated with methylprednisolone taken twenty one days post wounding. Note:- Collagen (c), epithelium (E), scanty blood vessel (Bv), H & E x 100.

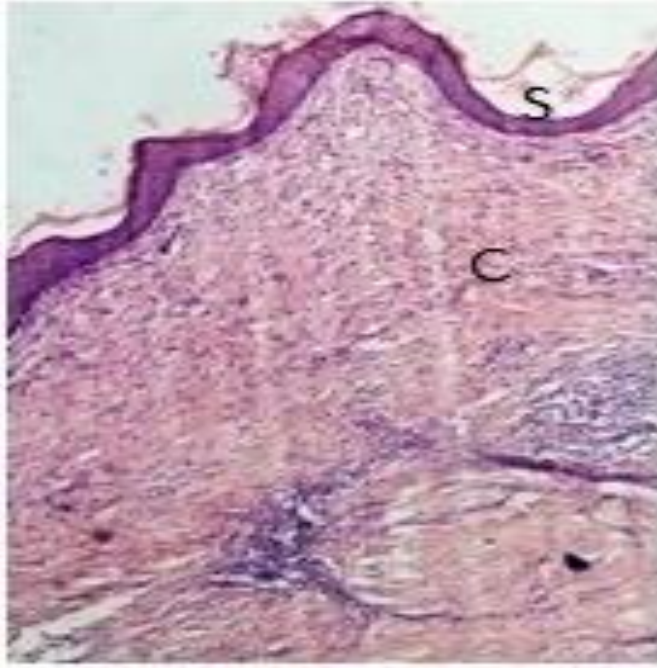


Plate LII- Photomicrograph of the skin of dog in (untreated) taken thirty days post wounding. Note:- Collagen (c), scar (S), H & E x 100.

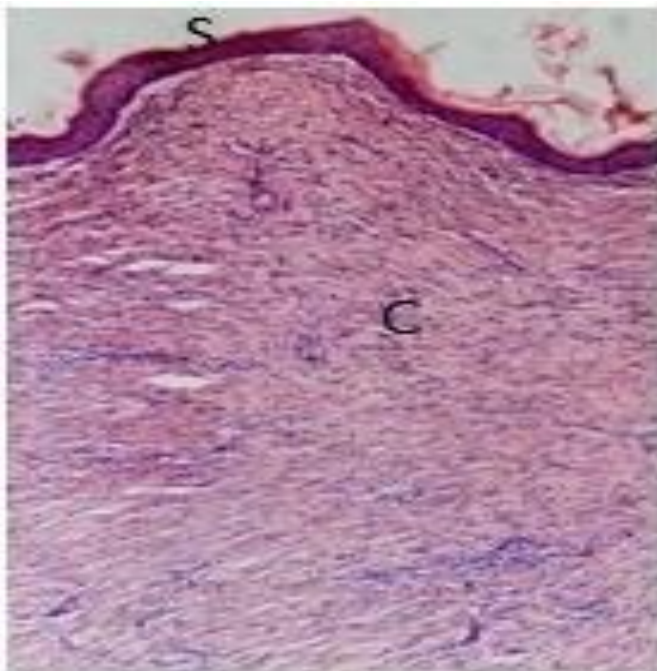


Plate LIII- Photomicrograph of the skin of dog treated with hydrocortisone taken thirty two days post wounding. Note:- Collagen (c), scar (S), H & E x 100.

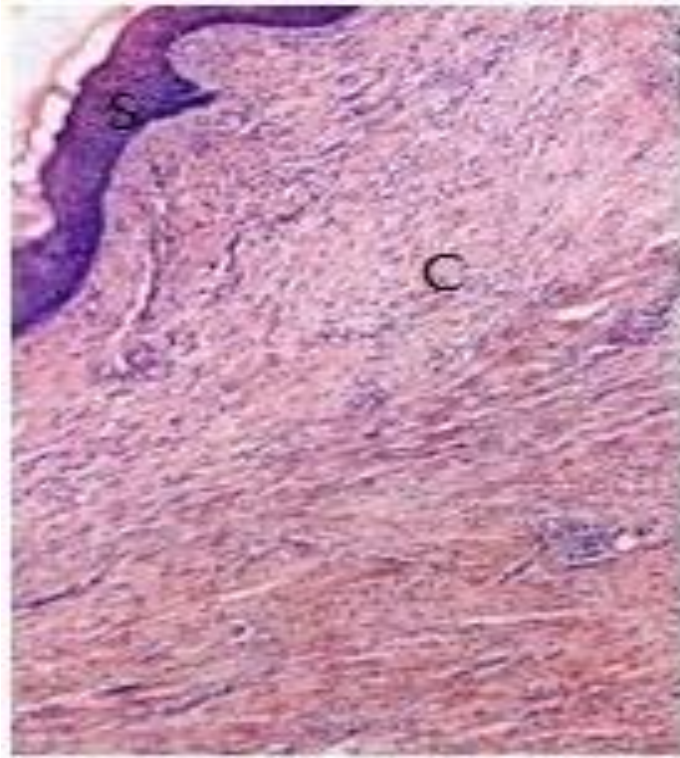


Plate LIV- Photomicrograph of the skin of dog treated with dexamethasone taken thirty days post wounding. Note:- Collagen (c), scar (S), H & E x 100.

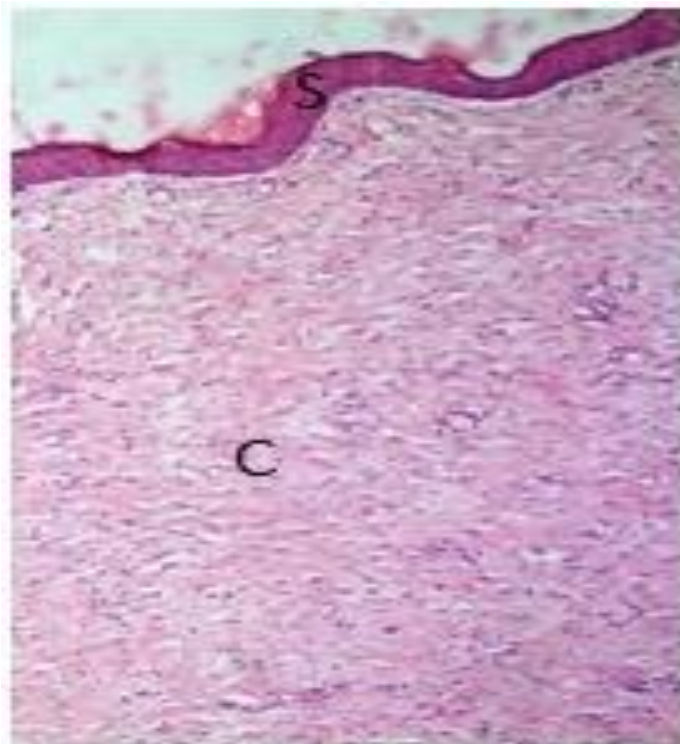


Plate LV- Photomicrograph of the skin of dog treated with methylprednisolone taken twenty five days post wounding. Note:- Collagen (c), scar (S), H & E x 100.



Plate LVI- Photomicrograph of the scar tissue of dog treated in control (untreated) taken thirty days post wounding. Note:- Collagen (c). VVG stains.

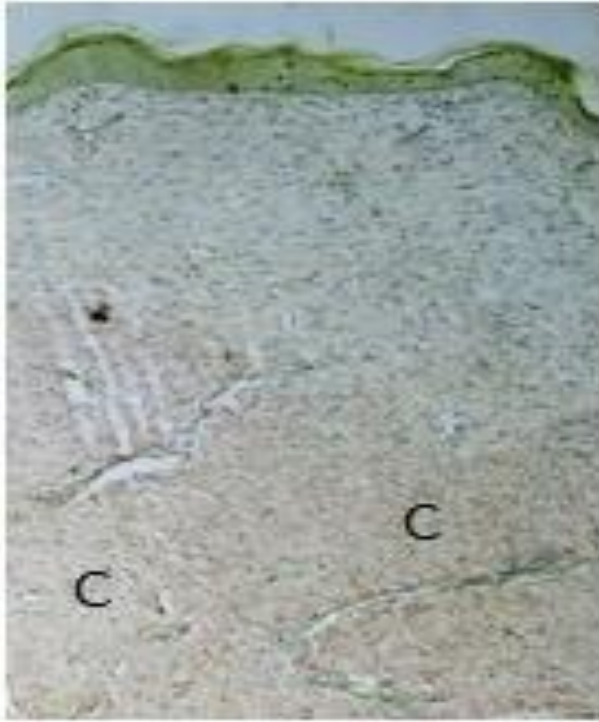


Plate LVII- Photomicrograph of the skin of dog treated with hydrocortisone taken thirty two days post wounding. Note:- Collagen (c), VVG stains.

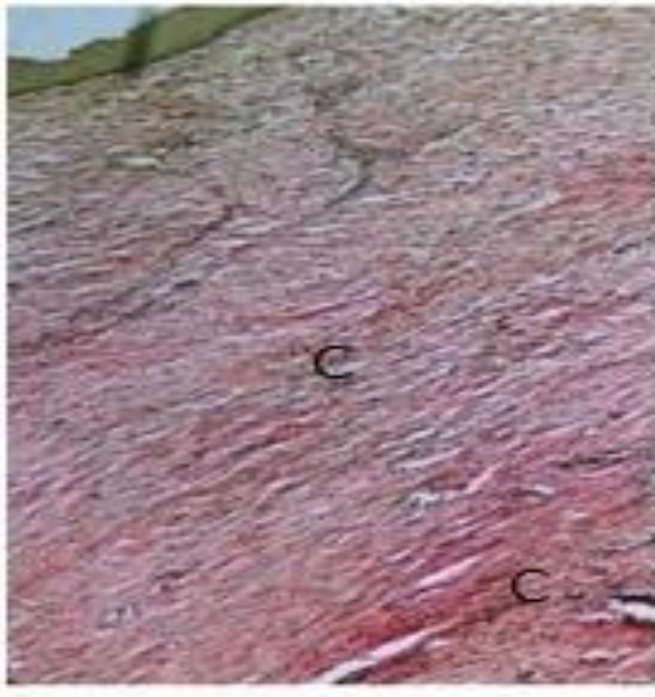


Plate LVIII- Photomicrograph of the skin of dog treated with dexamethasone taken thirty days post wounding. Note:- Collagen (c). VVG stains.

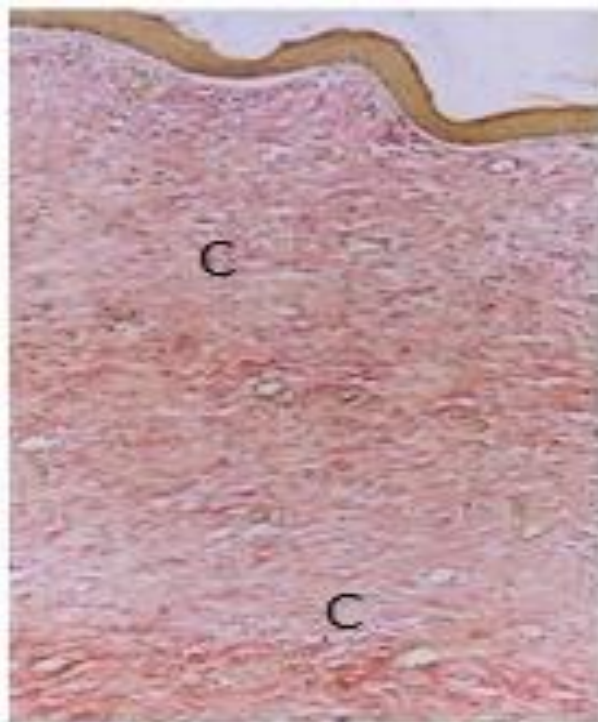


Plate LIX- Photomicrograph of the skin of dog treated with methylprednisolone taken thirty five days post wounding. Note:- Collagen (c). VVG stains.

CHAPTER 5

DISCUSSION

5.2 Fundamental Parameters

5.2.1 Haemogram

Eosinopenia as reported by Bjornson, *et al.*, (1985) and Haul, (2010) occurs with the use of steroids. Eosinopenia was also seen in this work with the use of steroids, however,

eosinopenia was more pronounced in the methylprednisolone and the hydrocortisone treated groups. The level of suppression was, however, not judged to be clinically significant. In this work band cells were more in the steroid treated groups than in the control group though all the values were within normal range. This justifies its use in the elderly and those with wasting conditions to enhance wound healing instead of making people obese as reported by Gray and Cooper, (2001). The hydrocortisone treated group had more band cells of the entire group (appendix 5). Steroids are recommended in hypersensitivity reactions and had been observed in the course of the experiment that, in the hydrocortisone treated group two out of the three dogs used showed signs of itching on day eight and day nine post-wounding just as all the dogs in the control group showed signs of itching on day three post-wounding. Though itching may be due to mast cell activities, since the degranulation of mast cells leads to the release of histamine in dogs (Riley, *et al.*, 2010), this may not be responsible for observation made in this study, since at therapeutic doses steroids are expected to stabilise mast cells and hence prevent degranulation.

Total plasma protein as recorded using refractometer in this study showed no statistical significant differences between the control and the steroid treated groups and within the steroid treated groups. However, there was a sharp decline in total protein concentration in all the groups by day 7 post-wounding, this was in line with Gray and Cooper, (2001) and Woodward, *et al.*, (2010), who reported that with frequent change of wound dressing, total plasma protein concentration may be depleted. This depletion as seen in this work was not significant which could likely be due to the small size of the wound.

5.2.2 Inflammation

Inflammatory processes starts with the injury itself (Anon^b, 2009) and this leads to infiltration of inflammatory cells into the wound site (Anon^h,2009; Gabriel *et al.*, 2009). From this study, wound size increased steadily from the day of wounding with severe infiltration of inflammatory cells in all the experimental groups by day 3 post-wounding. This increase in wound size could be due to inflammatory edema. However, Mahmut *et al.*, (2003) reported that more inflammation occurs in the steroid treated group than in the control group, which was contrary to our findings, where the control group had more inflammatory cells and more edematous. This is in line with Hemingwa, (1980) and Brander *et al.*, (1991) who reported that steroid reduces inflammation, inflammatory cells and edema. The methylprednisolone-treated group had the least cellular infiltration, while the hydrocortisone-treated group among the steroid treated groups had the most infiltration of cell, this could be due to the fact that hydrocortisone showed the least glucocorticoid effects (Martin, 2003). It is worth noting that, the anti-inflammatory drugs used in this experiment did not totally prevent edema in the steroid treated groups. Perhaps the use of the steroids prior to wounding may reduce edema, since it's been established that inflammatory process commence at the insult of the wound and that steroids block the inflammatory process. (Gabriel *et al.*, 2009)

5.2.3 Epithelialisation

Onset of epithelialisation occurred from day 5 post-wounding in the excisional wound of all the groups but from day 3 in the incisional wounds. The onset of epithelialisation as stated by Loning, *et al.*, (2004) and Gabriel, *et al.*, (2009), is from the wound edge as observed in this work.

In line with Fishman, (2009) epithelialisation can cover an area of 9cm^2 , which is even more than the wound dimensions (6cm^2) created in this work. Fishman, (2009) reported that epithelialisation occurs in all directions, though this was also true in this study, in addition we observed that epithelialisation was not uniform from all directions, but more from one direction (unilateral), that is, caudo-cranially. The non-uniform epithelialisation could be due to the skin cells arrangement.

Steroids mobilise amino acid and fat from cellular store for immediate energy release and enhance wound healing (Guyton and Hall, 2001). Histologically, methylprednisolone-treated group of all the experimental groups in both incisional and excisional wounds were the first to form epithelium on day 14, while by day 17 dexamethasone and methylprednisolone-treated groups were the only groups with epithelium. By day 21 all the experimental groups had epithelium histologically. This suggest superior wound healing properties by methylprednisolone and this could also mean that amino acids were more mobilised to the wound site in the methylprednisolone treated group followed by the dexamethasone, hydrocortisone treated groups and then the control group.

5.2.4 Wound contraction

The wound dimension at the beginning of the study after wound creation was observed to be more than the dimensions at complete epithelialisation in both the excisional and the incisional wounds of all the groups.

Wound dimensions increased from the day of wounding to the 10th day post wounding in the control group, day 12 in the dexamethasone and hydrocortisone treated group and day 17 in the methylprednisolone treated group. This was Contrary to O'leary *et al.*, (1998) and Stadelmann *et al.*, (2002) who stated that wound contraction was maximal between 5th-15th day. The initial increase in wound size could likely be due to inflammatory edema as also reported by Janice, (2002) who reported that wound size increases as necrotic tissue is debrided and wound size decreases as infection is controlled and healing occurs by epithelialisation, moreso, Joseph and Chris, (2007) observed that during 4 weeks before oxygen therapy mean wound area tended to increase in size.

5.2.5 Granulation tissue formation and collagenisation

Onset of granulation tissue formation is evident from 48 hours post-wounding (Golsen, 1989; Peacock and Kelman, 1990). This was also true in this study as granulation tissue formation was seen on the 3rd day post-wounding in all the 4 groups from the wound edges. By day seven, however, granulation tissue was observed to have been almost fully formed in both the excisional and the incisional wound of all the experimental groups, but relatively more in the dexamethasone treated group and the control group. This may explain why the control and the dexamethasone had almost same time of wound healing and also it may also mean that dexamethasone had insignificant suppressive effect of fibroblast compared to hydrocortisone and methylprednisolone.

The more collagen is been laid down, the more the granulation tissue gives less bright pink colour (Golsen, 1989). This was also seen in this work as the bright red colour of the granulation tissue on day 14 for the incisional wound becomes slightly deem by day 21.

Similarly for the excisional wounds. This was further confirmed with the histopathological slides.

5.2.6 Remodeling

Histologically from the scar, methylprednisolone-treated group had the most organised cells, followed by the dexamethasone and the hydrocortisone treated groups. The control group had the least organised cells in the scar. The organised cells in the methylprednisolone treated group could likely be due to the time the scar was taken or because it had the minimal edema throughout the work. Consequently, unlike the other groups methylprednisolone had uniformly distributed collagen tissue. The methylprednisolone treated group also had high deposit of collagen, which can mean more wound strength. The scar formed in the dexamethasone treated group had the thickest epithelium of all the groups. The hydrocortisone treated group which has the minimal deposit of collagen can be said to have the most flexible scar.

Anstead, (1998), reported that steroids increase the risk of wound healing delay and infection, and that steroids interfere with collagen synthesis, deposition and degradation, angiogenesis, contraction and epithelialisation. However in this work, collagen deposit was observed to be more in the steroid treated groups than in the control group. This may imply that the use of steroids at therapeutic doses reduce stress as reported by Brown, (2008).

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary and Conclusion

The administration of steroids at therapeutic dose for five days though had effects on wound healing process including; methylprednisolone had the best arrangement of collagen, early epithelisation and the thinnest scar. This could be recommended for facial, ocular and other cosmetic surgery; dexamethasolone produced the thickest scar and therefore could be recommended for use in wounds where high strength is required like herniorraphy and; dexamethasone and methylprednisolone-treated dogs showed absence of itching during wound healing when compared to the control and hydrocortisone-treated dogs. These could be used in management of general wounds, but was not of any statistical significance.

6.2 Recommendations

This study was able to bring out the following:

1. Hydrocortisone which is generally prescribed in hypersensitivity reactions was seen in this work to have only delayed itching in the treated group but not completely prevent it as in the dexamethasone and methylprednisolone treated group.
2. The wound size at the end of this study appeared to be smaller scar in the groups that showed signs of itching, could it be that mast cells have any significant role in wound contraction and are responsible for the itching?.
3. Finally, I will like to recommend that more work be done in this aspect to improve on quality of wound healing with the use of steroids. Investigation needs to be conducted at the molecular and micromolecular levels to ascertain the effects of

these steroids, more so their use prior wounding and at doses other than the therapeutic dose.

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Appendix 1. Mean Excisional Wound Area

Days Post surgery	Control	Dexamethasone Treated	Hydrocortisone Treated	Methylprednisolone Treated
1	6.00	6.00	6.00	6.00
3	9.89	8.72	11.56	9.57
5	9.50	10.53	11.38	9.78
7	8.12	11.75	11.91	9.63
10	10.34	10.88	11.01	12.21
12	7.23	11.97	12.21	12.06
14	5.62	8.52	11.35	13.00
17	4.99	6.62	10.88	13.87
19	3.91	5.15	6.81	8.54
21	3.84	4.98	5.43	6.85
24	4.09	4.63	5.37	5.80
26	4.20	4.03	5.00	5.68
28	4.86	3.86	4.63	5.40
31	3.52		4.48	4.96

33	3.52	4.17	4.89
35		3.87	4.55
38		3.72	4.25
40		3.55	4.00
42			3.98

Appendix 2. Mean Incisional Wound Area

Days Post-surgery	Control	Dexamethasone Treated	Hydrocortisone Treated	Methylprednisolone Treated
1	420	420	420	420
3	600		475	549
5	520		585	461
7	631		616	751
10	610		581	455
12	344		564	490
14	282		538	434
17	275		495	381
19	243		314	358
21	228		265	301
24	232		239	275
26				261
28				254
31				332
33				317

Appendix 3. Mean Excisional Wound Area Epithelisation

Days Post-surgery	Control	Dexamethasone Treated	Hydrocortisone Treated	Methylprednisolone Treated
3				
5	+	+	+	+
7	92	168	121	110
10	144	224	181	171
12	183	271	212	228
14	226	298	218	247
17	233	311	227	240
19	249	337	231	265
21	250	347	265	268
24	302	365	294	292
26	340	380	297	331
28	343	386	307	349
31	350		326	351
33	352		342	356
35			344	364
38			352	376
40			355	382
42				398

Appendix 4. Mean Incisional Wound Area Epithelization

Days Post-surgery	Control	Dexamethasone Treated	Hydrocortisone Treated	Methylprednisolone Treated
3	+	+	+	+
5				
7	61	104	48	64
10	92	209	78	100
12	109	228	145	171
14	188	232	164	180
17	192	240	192	212
19	210	242	212	217
21	223	249	225	226
23	232	256	234	236
26		268	241	247
28			249	266
31			254	302
33				317