Wound healing potential of *Salix acmophylla* in full thickness skin wound using rabbit model

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(2013-V-250-M)

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THESIS

Submitted to
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University of Agricultural Sciences &Technology of Kashmir in partial fulfillment of requirement for the award of the degree of

Master of Veterinary Sciences
(Veterinary Surgery and Radiology)

2016

DEDICATED

TO MY

LOVELY PARENTS

AND

RESPECTED MAJOR ADVISOR

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Veterinary Surgery and Radiology
Shuhama Campus, Srinagar-190006

Certificate – I

This is to certify that the thesis entitled, **"Wound healing potential of *Salix acmophylla* in full thickness skin wound using rabbit model"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Sciences (Veterinary Surgery and Radiology)**, to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Zahid Rahim Malik (2013-V-250-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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ABSTRACT

The indigenous herbal drug preparations contribute sufficient increasing interest in wound healing alternative to conventional antiseptic, antibiotic and anti-inflammatory agents. As per the Unani system of medicine the leaves of willows give "cold dry" effect while the flowers display "cold wet" effect. *Salix* plants contain a wide variety of compounds called phytochemicals, mainly described as those compounds having medicinal properties. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely. From the review of literature, it is clear that experimental wound healing potential for the *Salix acmophylla* leaves selected seems to be fewer and needs further investigation. This necessitates the exploration of this herb for its efficacy in wound healing. In present study, four full thickness excisional skin wounds (1.5×1.5 cm^2), 2.5 cm apart from each other were created (dorsal spine of thoraco-lumbar region) on each of 18 rabbits (three groups having six animals) of either sex and weighing 1.8 to 2.5 kg under standard anesthetic protocol to evaluate the wound healing potential of *salix acmophylla* leaves ointment and their response on clinico-physiological, haemato-biochemical, kidney function

test, bacterial viable count, gross and histomorphological parameters. The total number of wounds evaluated in the study were 72 and thus each treatment groups was evaluated on 24 rabbits. The experimental study was conducted in three groups (I, II and III). Group I animals were treated with sterile normal saline solution (negative control). Groups II and III rabbits were dressed topically with thin layer of 5% povidone iodine and 5% *salix acmophylla* leaves ointment, respectively. Povidone iodine treated wounds were used as positive control. Rabbits were evaluated for change in rectal tempereature (RT), heart rate (HR), respiration rate (RR), total erythrocyte Count (TEC), differential leukocytic count (DLC), hemoglobin, packed cell volume (PCV), glucose, albumin, total protein (TP), creatinine level, blood urea nitrogen (BUN), wound size, percentage healig, total bacterial count level, photographic evaluation, gross and histomorpholigical examination.

No physiological side effects and other complications were observed in any group. The salient observations of the photographic observation suggest that all the excisional wounds aseptically created were almost equidimensional on day '0' (immediately after surgery). It was interesting to note on day 7 after post-wounding that there was appreciable reduction in size of wound treated with *salix* and povidone iodine as compared to control groups. Furthermore, complete filling of the wound with granulation tissue without scab and demonstrable wound contraction was noticed in *salix* on day 14 post-wounding than povidone iodine and normal saline solution groups. Complete epithelialization and closure of *salix* and povidone iodine treated wouds could be distinguished on day 21 and percentage healing 100.00% and 95.66% respectively were observed, whereas in normal saline solution complete healing was noticed in only 61.00% of wounds. Grossly, wounds treated with 5%, *salix acmophylla* leaves ointment significantly accelerated the rate of wound healing compared to wounds treated with sterile normal saline solution or dressed with povidone iodine ointment. Histological analysis of healed wounds confirmed the gross observations. Wounds dressed with 5%, *salix acmophylla* leaves ointment showed markedly less scar width at the wound enclosure with large amounts of fibroblasts proliferation, more mature and densely packed collagen and angiogenesis compared to wounds dressed with sterile normal saline solution. Overall results indicated that wounds dressed with *salix acmophylla* leaves ointment (group III) showed considerable signs of full thickness skin wound healing and significantly ($p<0.05$) healed earlier in 17.83 days followed by povidone iodine treated wounds (group II) in 19.00 days than wounds dressed with sterile normal saline solution (group I) in 20.83 days. These results strongly document the beneficial and significant effects of *salix acmophylla* leaves ointment for the acceleration of wound healing in rabbits.

Key words : Rabbit, full-thickness wounds, 5% *salix acmophylla* leaves ointment, 5% povidone iodine ointment, wound healing.

Signature of Student   Signature of Major Advisor

Dated: ____________   Dated: _________________

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Zahid Rahim Malik

Place : Shuhama
Dated :

CONTENTS

Abbreviations

%	:	Percent
@	:	At the rate of
µ	:	Micro
µg	:	Microgram
^{0} C	:	Degree celcius
ANOVA	:	Analysis of variance
b.wt	:	Body weight
C K	:	Creatine kinase
CAT	:	Catalase
CDT	:	Conventional drug therapy
Cm	:	Centimeter
CNS	:	Central nervous system
D	:	Day
dl	:	Deciliter
DLC	:	Differential leukocyte cell count
EDTA	:	Ethylene diamine tetra acetic acid
et al	:	et alia (and others)
Fig	:	Figure
g	:	gram
g/L	:	gram per liter
Hb	:	Haemoglobin
HR	:	Heart rate

i.e	:	id est (that is)
i.m	:	Intramuscular
mg	:	milligram
ml	:	millilitre
N	:	Neutriphils
NSAIDs	:	Non-steroidal anti-inflamatory drugs
NSS	:	Normal saline solution
$P>0.05$	:	Non- Significant at 5%
$P<0.05$	:	Significant at 5%
RBCs	:	Red blood corpuscles
RR	:	Respiratory rate
RT	:	Rectal temperature
SE	:	Standard Error
SPSS	:	Statistical package for social sciences
U	:	Unit
vol	:	Volume
w/v	:	Weight/ volume

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Chapter-1

INTRODUCTION

A wound may be defined as disruption of the cellular or anatomical continuity of the normal organ structure (Bennet,1988). It is a common and frequent reason for seeking veterinary attention. The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and moist environment to restore the anatomical continuity and function of the affected part (Pierce and Mustoe, 1995; Begum and Nath, 2000). Cutaneous wound repair involves migration, infiltration, proliferation, and differentiation of several cell types like keratinocytes, fibroblasts, endothelial cells, macrophages and platelets which culminates in an inflammatory response, the formation of new tissue and wound closure (Phillips *et al.*, 1991; Barrientos *et al.,* 2008).

Veterinary practitioners often encountered animals with traumatic wounds that are infected, too large to close immediately or both. The way in which wounds are managed can affect the rate of healing, the time to return to normal function, the final cosmetic appreance, and hence the satisfaction of customers (Liptak, 1997). Despite some recent advances in understanding its basic principles, problem in wound healing continues to cause significant morbidity and mortality (Peacock and Cohen, 1990).

The management of wounds depends on the stage of wound healing and can include irrigation, mechanical and chemical debridement, use of antiseptic and antimicrobials, adherent and non-adherent dressings, and miscellaneous topical applications. In many cases, the best way to treat such wounds is to manage them as an open wound (Yamanda, 1983; Lee *et al.,* 1984). Second intention wound healing occurs by wound contraction, re-epithelization, and finally production of granulation tissue (Swaim *et al.,* 2001). Within 5 to 7 days after injury, granulation tissue develops from underlying connective tissue to cover a wound (*Lee et al.*, 1987; Layton, 1993).

Medical management of wound includes administration of drugs either locally (topical) or systemically (oral and parenteral) in an attempt to aid wound repair (Savanth and Shah, 1998). The topical agents used include antibiotics and antiseptics (Chulani, 1996), de-sloughing agents (chemical debridement e.g. hydrogen peroxide, eusol and collagenase ointment) (Savanth and Mehta, 1996), wound healing promoters (e.g. Tretinoin, *Aloe vera* extract, honey, comfrey, benzoyl peroxide, *Chamomilia* extract, dexpanthenol, tetrachlordecaxide solution, clostebol acetate and experimental cytokines). Various growth factors like platelet derived growth factors, macrophage derived growth factors, monocyte derived growth factors (Mather *et al.,* 1989) etc. are necessary for the initiation and promotion of wound healing. Many substances like tissue extracts (Udupa *et al.*, 1991), vitamins and minerals and a number of plant products (Dahanukar *et al.*, 2000) have been reported by various workers, to possess pro-healing effects.

A commonly used antimicrobial agent is povidone-iodine (Betadine), a complex of iodine, the bactericidal component, with polyvinylpyrrolidone (povidone), a synthetic polymer. The most common commercial form is a 10% solution in water yielding 1% available iodine. Synthetic or conventional drugs are synthesized chemically in the laboratory. Different conventional antiseptics used in a concentration, sufficient to control the septic process, adversely affect the immature cellular repair process (Heinz, 1976). Antibiotics are commonly used topically for preventing bacterial colonization. These antibiotics agents although check the infection and thus promote early wound healing, have certain limitations that may cause tissue destruction by irritation or allergy, non-availability and high cost (Nagesh *et al.,* 1999). Indiscriminate use of antibiotics has a threat of super-infection and also the emergence of resistant microorganisms (Myers *et al*., 1980). Consequently there exists a need for new agents which may be useful in proper wound management. In this direction a number of herbal products are being reported on the use of various indigenous medicinal plants to posses wound healing, anti-inflammatory, antibacterial, antifungal and analgesic properties and have been used for therapeutic purposes (Bhatnagar *et al.,* 1961; Shukla, 1967; Kumar *et al.,* 1996). The indigenous herbal drug preparations contribute sufficient increasing interest in wound healing alternative to conventional antiseptic, antibiotic and anti-inflammatory agents (Ahmed *et al.,* 1995). It is therefore, important that the dressing used should not promote the growth of harmful bacteria and at the same time it should not have deleterious effect on the tissue. Extensive research has been carried out in this field and many topically applied agents have been used to treat open wounds, but most products investigated in domestic animals either do not affect wound healing or inhibit rather that enhance it (Lee *et al.,* 1987; Swaim *et al.,* 1993; Swaim, 1997; Sardari *et al.,* 2006). Therefore, products selected to create a healing environment must be chosen thoughtfully and scientific rationale must support their use.

The over usage of synthetic drug resulting in higher incidences of adverse drug reactions have provoked mankind to go back to nature for safer remedies. Nature provides a wide variety of plants that contain medicinal properties. The powerful ingredients found in the stems, leaves, roots, flowers, and seeds of certain plants have natural healing properties that have been found to cure various diseases. Herbal medicinal plants (also called phytomedicinals) can be administered as the whole plant or plant parts or by extracting one or more ingredients with solvents to yield tinctures, tea or other extracts. Herbal medicines are the outcome of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal drugs being much less expensive than their synthetic counterparts also have better cultural acceptability, better compatibility with the human body and minimal side effects (Pal and Shukla, 2003, Venkatesh *et al.,* 2003). The herbal drugs have been used throughout the world and have received greater attention in recent times, because of their diverse nature of curing diseases, safety and tolerance compared to conventional drugs. The utilization of herbal drugs is on the flow (Kamboj, 2000). Herbal drugs have been used for wound healing in either crude form or in the form of different extracts. According to the World Health Organization (WHO), the use of herbal drugs throughout the world has increased tremendously (Pal and Shukla, 2003). In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases (Biswas *et al.*, 2003). The knowledge of uses of plants transmitted from one generation to the next (Dexit and Pandey, 1984). Plants are exploited in many ways such as food, fodder, fuel wood, timber wood, medicinal, etc (Hussain and Khaliq, 1996). The therapeutic use of medicinal plants has gained a considerable energy in the past few decades. It shows that there is a huge contact of human life with local flora as well as local flora influence human beings. Medicinal plants are also important for the livelihoods of deprived communities all over the world.

Continual exposure to stressful conditions generates free radicals, which may over power the inbuilt protective mechanisms and cause tissue damage. There are reports that plants possessing free radical scavenging activity are known to have anti inflammatory, anti tumour, wound healing and many other activities. Of these, *Salix* spps. is distributed throughout tropical and sub tropical parts of India, Sumathra and Java (Kirtikar and Basu, 1975). Species of *Salix* found in almost all regions of Kashmir as *Salix acmophylla.* Willow and "bains/ Wir/ Veer Kani" respectively, are common English and vernacular names for a number of sister trees of the genera *Salix* representative of family Salicaceae. These are fast growing and yet medium-sized deciduous trees. It grows primarily in the cool, fertile, irrigated lands as it requires larger quantities of water, though it can withstand cold winter frost (Rather *et al.*, 2010). They are of enormous ecological and economic importance (Argus, 1997). As per the Unani system of medicine the leaves of willows give “cold dry” effect while the flowers display “cold wet” effect. *Salix* plants contain a wide variety of compounds called phytochemicals, mainly described as those compounds having medicinal properties. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely (Zarger *et al.*, 2014). *Salix spps*. (Willows) are the source of the natural precursor to asprin, salicylic acid, found in leaves and bark (Pojar and

Mackinnon, 1994). The bark and leaves can be pounded and applied to wounds as healing agents (Moerman, 1998). The active ingredient of the *salix* bark is called salicin. Salicin hydrolyzes in aqueous media to glucose and salicylic alcohol (saligenin). *Salix* spps. have abundant watery bark sap, which is heavily charged with salicylic acid. Besides, salicin, it contains flavonoids and proanthocyanidins, which are potent wound healing agents (Zarger *et al.,* 2014).

Experimental surgery offers the freedom of biopsying wounds, doing histopathological and histochemical studies, which facilitates objective evaluation of progress of wound healing. The Indian traditional system of medicine is based on pragmatic facts of the observations and the experience over millennia. More than 1200 diseases are mentioned in different classical texts. Traditional medicine, being a significant element in the cultural patrimony, still remains the main choice for a large majority of people for treating various diseases and ailments. From the review of literature, it is clear that experimental wound healing potential for the *Salix acmophylla* leaves selected seems to be fewer and needs further investigation. This necessitates the exploration of this herb for its efficacy in wound healing. Keeping all these in mind the work was designed with the following objectives:

Objectives of the Investigation:

1. To investigate the efficacy of *Salix acmophylla* leaves ointment as full thickness skin wound healing agents.
2. To study the effect of *Salix acmophylla* leaves ointment on kidney function in rabbits.

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Figure 1. ***Salix acmophylla*** **plant**

Figure 2. ***Salix acmophylla*** **leaves**

Chapter – 2

REVIEW OF LITERATURE

2.1. ANATOMY OF SKIN

Skin is the outermost tissue of the body and the largest organ in terms of both weight and surface area. It consists of many components and adnexa, including hair follicles, sebaceous glands, and sweat glands. The main function of skin is to act as a barrier against environmental aggressions. The skin is formed by three anatomically, functionally, and developmentally distinct layers: epidermis, dermis, and hypodermis. The epidermis is in fact a -layered epithelium extending from the basement membrane that separates it from the dermis to the air. Except for the basement membrane, it is virtually devoid of extracellular matrix (ECM). Progenitor cells are located on the basement membrane and undergo continuous self-renewal and differentiation to keratinocytes. The keratinocytes migrate towards the surface of the skin where they eventually get sloughed off. While this process occurs, keratinocytes undergo terminal differentiation and maturation (Blitterswijk and Thomsen, 2008). As they approach the skin surface, they form a keratinized layer of dead cells which confers the main barrier properties of the skin (Harvey, 2005).

Below the epidermis is the dermis, the thickest of the three layers of skin which accounts for most of the skin's mechanical properties and resilience. The dermis is a connective tissue comprised of ECM, fibroblasts, vascular endothelial cells, and skin appendages (hair follicles, sweat glands, etc.) Fibroblasts secrete collagen and elastin, providing mechanical strength and elasticity to the skin, respectively. The hypodermis underneath the dermis is composed of adipose tissue, which functions as insulation and cushioning between the skin and other skeletal structures, like bone and muscle (Harvey, 2005).

2.2. Wound and their classification

A wound is an injury characterized by the disruption of normal continuity of body structure (Waldron and Trevor, 1993). According to Silver (1982) wound is a situation in which cells are at the limits of their power of survival and a very slight modification of the microenvironment can render the tissue either hostile to cell or can enhance cellular activity. In everyday pathology, wounds remain a challenging clinical problem, with early and late complications presenting a frequent cause of morbidity and mortality (Alonso *et al.*, 1996, Natarajan *et al.*, 2000). Wound composed of a wall and its contents, contents may be damaged tissue debris and blood clots. Wound wall is said to be composed of three zones actively involved in wound repair, viz: central zone (composed of dead tissue), ischemic zone (surrounding the central zone) and a zone of reaction (surrounding the ischemic zone characterized by acute inflammatory).

There are many different ways in which wounds can be classified (Swaim, 1980; Frank, 1981, Bright and Probst, 1993; Singh and Singh, 1993, Ansari, 2015): 1. First classification: i. Simple: if no deeper tissues are involved. ii. Compound: when muscles, nerves, tendons and bone etc. are involved. 2. Second classification based on morphology of the wound: A. Open or external wound: in which there is discontinuity in the skin and other covering tissues to a varying depth. B. Closed wound/internal wound: in which there is no break in the continuity of tissue surface (skin) but in which deeper tissues (subcutaneous tissues, muscles, and nerves) are damaged to a varying degree. For example: contusion (subcutaneous), bruise, haematoma. 3. Third classification: based on contamination: i. Clean/ sterile or aseptic wound: a wound made under aseptic condition e.g., surgical wound. ii. Clean-contaminated wounds: in which there is minimal contamination comes from the environment, surgical team, or patient's skin surrounding the wound. iii. Contaminated wound: Contaminated wounds, a wound in which there is the presence of foreign debris /microorganisms. iv.

Infected wound or septic wound: wound with bacterial growth showing local changes like suppuration, cellulitis. In clean, clean-contaminated and contaminated wounds bacterial load may be less than 105 organisms per gram of tissue. In Infected wounds contains more than 105 organisms per gram of tissue. Golden period is the first 6-8 hours between wound contamination at injury and bacterial multiplication greater than 105 organisms per gram of tissue. 4. Fourth classification based on duration: Class I: Clean, non-infected surgical/incised wound- 0 to 6 hours duration, no signs of inflammation, Class II: Significant contamination, contaminated wound - less than 6 to 12 hours duration old, no evidence of infection, Class III: Gross contamination, infected wound-12 or longer hour's duration, non purulent inflammation. 5. Fifth classification based on thickness of the wound: I. Superficial wounds, involving only the epidermis and the dermis up to the dermal papillae, II. Partial-thickness wounds, involving skin loss up to the lower dermis. III. Full-thickness wounds, involving the skin and the subcutaneous tissue. IV. Deep wounds, including complicated wounds (viz., with laceration of blood vessels and nerves), wounds penetrating into natural cavities, and wounds penetrating into an organ or tissue:

A. Open or external wound: in which there is discontinuity in the skin and other covering tissues to a varying depth. 1. Incised wound: are caused by sharp cutting instruments such as knives, scalpels, fragments of glass etc. with minimum loss to tissue, edges are regular, bleeds freely and painful. In this wound, edges are smooth and causing minimal damage to the surrounding tissues. 2. Lacerated wounds: these are caused by tearing of tissues e.g., barbed wire injury. In this wound, edges are irregular and torn. 3. Penetrating wound: these types are deep wounds caused by long pointed objects like daggers, pointed knives and may communicates with abdomen, thorax, larynx, joints, etc. for example: stab wounds.4. Punctured wound: these types of wounds are created by sharp pointed objects like nails cause punctured wounds. e.g., Punctured wound on foot due to gathered. 5. Perforating wound: is characterized by two opening, one of entrance and other of exit. 6. Avulsion/Evulsion wound: these wounds are characterized by tearing of tissues from their attachments like avulsion of horn, hoof etc.7. Abrasion: damage of superficial layers of skin produced by friction and pressure results. 8. Gunshot wounds: these types of wounds are produced by different types of firearms. 9. Ulcerative wound: is one in which there is no tendency to heal. 10. Granulating wound: is one in which there is a tendency to heal within expected time. 11. Bite wound: which are caused by snake, dog or wild animals bite with significant degree of tissue damage. 12. Virulent wound: which are caused by bacteria or virus leading to formation of pustules or vesicles for example foot and mouth disease, anthrax.

B. Closed wound (internal subcutaneous or interstitial): in which there is no break in the continuity of tissue surface (skin) but in which deeper tissues (subcutaneous tissues, muscles, and nerves) are damaged to a varying degree. For example: contusion, abrasions, wheal.

a. Contusion (subcutaneous): are caused by blunt objects and results in damage to subcutaneous tissues without breaking the continuity of the skin surface. It is classified into first, second and third degree according to the extent or severity of the injury. 1. First degree contusions (bruises): echymosis, as a result of ruptured capillaries in the skin and subcutaneous tissues and dissemination of blood through the intercellular spaces without formation of gross collection of blood, leading to reddish blue or purplish coloration of skin (non-pigmented skin). 2. Second degree contusions (hematoma): collection of blood in abnormal cavity usually caused by superficial vein injuries and frequently seen subctaneously, submucously, or subcapsular in large parenchymatous organs. 3. Third degree: is the major damage of tissue leading to gangrene formation. b. Abrasions: when there is injury to the superficial layer of the skin or mucous membrane is damaged but has not extended through all the layers. c. Wheal: is a bleb in skin internal in nature with no extravasation of blood.

2. 3. Wound Healing

Normal wound healing is a dynamic and complex process involving coordinated interactions between diverse immunological and biological systems.

Healing is a process whereby the body destroys and removes the irritants and returned the part as near a normal functional state as possible. It is closely associated with inflammation and works in the directions simultaneously e.g., the removal of the devitalized tissue and the active regeneration of the tissue to restore the functional activity of the damaged tissue by fibrocollagenous proliferation and a surface covering. Wound healing is a complicated interaction of cellular (platelets, endothelial cells, macrophages, fibroblasts), and sub cellular (growth factors and cytokines) components. Wound healing involves a cascade of carefully and precisely regulated steps and events that correlate with the appearance of various cell types in the wound bed throughout the distinct phases of the healing process (Hunt *et al.,* 2000; Broughton and Attinger, 2006). Wound healing involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in four phases namely:

1. **Stage of hemorrhage**: initial hemorrhaged results in the formation of clot which occupies the defect in the tissue. The clot cosists of a fibrin framework containing trapped blood elements. The cut vessels are also sealed by contraction. The adjacent cut margins of the wound become glued together by a thin layer of coagulum. Although blood flow within the wound is often impaired due to destruction of the blood vessels, it is elevated in the areas immediately adjacent to the wound, and local inflammatory agents (activated complement, histamine, etc.) increase vascular permeability leading to plasma extravasation and the generation of a fibrin matrix, also causing swelling and redness (Harvey, 2005). This matrix is rapidly invaded by neutrophils, followed by monocytes and other immune-competent cells to remove dead tissue and control infection (Blitterswijk and Thomsen, 2008).

2. **Stage of hyperemia (Phase of traumatic inflammation):** This inflammatory phase typically lasts for the first 4 days (Rhett *et al.,* 2008). This phase is characterized by an acute inflammatory reaction. The severity of this phase is related to the degree of trauma to the tissue and shows vascular and cellular responses. When a wound is made some of the cells are traumatized and

destroyed, resulting in release of potassium ions, peptides e.g., leukotraxine, histamine and histamine like substances which are supposed to be responsible to cause vasodilatation, lack of oxygen to the tissue after injury and metabolic end products in such conditions, increase the local acidity which sustains vasodilatation. There is an increase in blood and lymphatic flow. This further show exudation and increased migration of leukocytes, to the area through permeable capillary membrane. Phagocytosis and enzymatic digestion of the devitalized tissue begin and the phagocytosed bacteria, foreign bodies and dead tissues are gradually removed as pus, through the lymphatic channel, which is the chief route. This phase is known as phase of traumatic inflammation.

The inflammatory phase prepares the zone for healing and immobilizes the wound by causing it to swell and become painful, so that movement becomes restricted. At the tissue level, increased vascular permeability and the sequential migration of leukocytes into the extravascular space characterize inflammation (Cohen *et al.*, 1999). Within hour of injury, the release of Endothelial Growth Factor (EGF), transforming growth factor (TGF-β), Fibroblast Growth Factor (FGF) act to stimulate epithelial cell migration and proliferation through the acts of reproduction and mitosis, resulting in the start of re-epithelialization. Platelet-Derived Growth Factor (PDGF), attracts neutrophils to the wound site to remove contaminating bacteria, foreign particles, and damaged tissue (Hantash *et al.*, 2008) and their main function is to prevent infections (Broughton and Attinger, 2006). With the help of transforming growth factor (TGF-β), monocytes are attracted to the wound site and converted into macrophages. These cells have a longer life than neutrophils and play an important role in augmenting the inflammatory response and tissue debridement. Macrophages provide an abundant reservoir of potent tissue growth factors (Endothelial Growth Factor (EGF), TGF-β, Fibroblast Growth Factor (FGF), and Vascular Endothelial Growth Factor (VEGF), and PDGF) and pro-inflammatory cytokines (IL-1 and IL-6) which activate keratinocytes, fibroblasts, and endothelial cells (Hunt et al., 2000; Broughton and Attinger, 2006). Therefore, inflammation is an essential phase in the healing process because it plays an important role in fighting against infection. Once the inflammation decreases due to the action of neutrophils and macrophage, their number is reduced and, as a result, the proliferation phase is initiated.

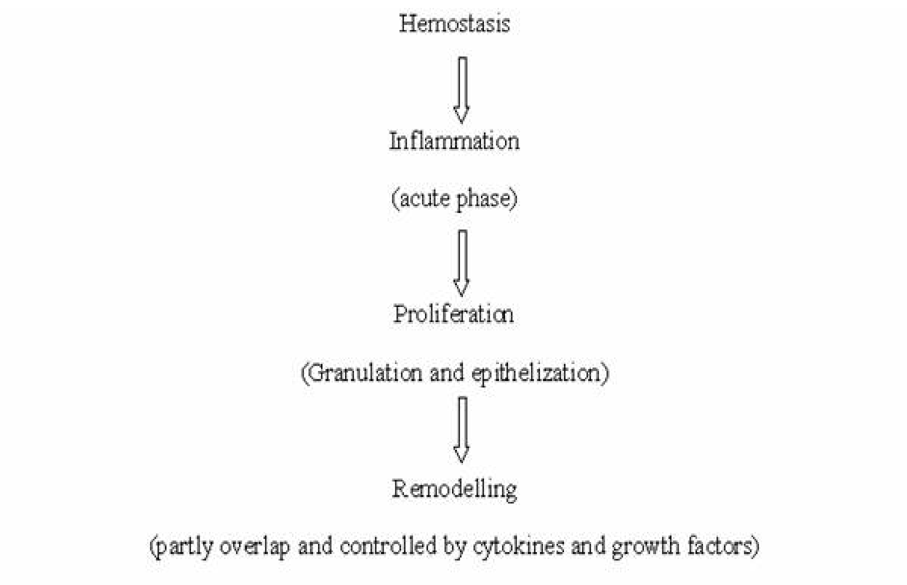
3. Stage of granulation (proliferation phase): it is characterized by fibroblast migration, deposition of new ECM and granulation tissue formation. The WBC that has acculumulated in the wound area give rise to monocellular non-granular amoeboid cells known as polyblast, which appear to the capable of being transformed into fibroblasts the connective tissue cells. Fibroblasts migrate into the wound in response to TGF-β and PDGF; there, they proliferate abundantly and produce matrix proteins as hyaluronan, fibronectin, elastin, proteoglycans, and type I and type III procollagen (Ramasastry, 2005; Witte and Barbul, 1997; Robson *et al.,* 2001). Fibroblasts begin to secrete collagen, gradually replacing the fibrin matrix. As more collagen is deposited and undergoes cross-linking, the mechanical strength of the wound increases. Collagens play a key role in wound healing since act as a base for the intracellular matrix formation within classical model of wound healing is divided into three sequential phases which overlap in the time and space: inflammation, proliferation, and maturation and remodeling. Migratory cells use this matrix as a bridge to crawl across and platelets adhere to it and secrete factors such as PDGF), EGF, TGF-β, FGF, and Vascular Endothelial Growth Factor (VEGF) (Heldein and Ostman, 1996; Midwood *et al.,* 2004). Unwounded dermis contains 80% type I and 20% type3 collagen, whereas wound granulation tissue expresses 40% type III collagen. At the end of the first week, an abundant accumulation of ECM supports cell migration, which is essential for the repair process (Broughton and Attinger, 2006; Ramasastry, 2005; Witte and Barbul, 1997; Robson *et al.,* 2001; Velnar *et al.,* 2009). Moreover, macrophages provide a continuing source of growth factors necessary to stimulate angiogenesis and fibroplasia. Fibroblasts produce the new ECM necessary to support cell in growth and blood vessels carry oxygen and nutrients necessary to sustain cell etabolism (Singer and Clark, 1999). The structural molecules of the newly formed ECM contribute to the formation of granulation tissue by providing a scaffold or conduit for cell migration. The process of angiogenesis occurs concurrently with fibroblast proliferation when endothelial cells migrate to the area of the wound. ese endothelial cells are stimulated by FGF to proliferate and release angiogenic growth factors, such as VEGF, which are responsible for the initiation of this process. Under hypoxic conditions, endothelial cells from uninjured blood vessels are chemo-tactically attracted to the found crawling through the ECM in order to form a network of new capillaries (Witte and Barbul, 1997; Robson *et al.,* 2001; Velnar *et al.,* 2009; Phillips, 2000). Capillary buds of endothelial cells grow from the existing blood vessels. Initially these sprouts remain solid and undergo canalization and by anastomosis with their neighbours form a series of vascular loops. These newly formed vessels leak protein and the tissues fluid formard a very suitable media for fibroblastic growth. Presence of protruding capillary buds gives the tissue a granular appearance so this newly formed tissue is called granulation tissue. The granulation tissue is formed from the base of the round and appears deep red. It is very fragile and slightest trauma causes bleeding. It does not contain nerves so is without sensation. This “granulation tissue” is observed between 5–20 days (Harvey, 2005).

Once the necessary amount of oxygen and nutrients re achieved at the wound site, angiogenesis ceases and blood vessels hat are no longer needed die by apoptosis (Ilan *et al.,* 1998). Re-epithelialization of founds begins within hours after injury. The release of growth factors stimulates epithelial cell proliferation and migration through the new tissue. During re-epithelialization, basement membrane proteins appear in an ordered sequence starting from the edges of the wound. Fibroblasts may also differentiate into myofibroblasts that express α-smooth muscle actin, which causes the wound to contract, thus reducing the wound area that needs to be closed by cell proliferation. Vascular endothelial cells and capillaries invade through a process of angiogenesis extending from nearby healthy tissue, as well as from the recruitment of endothelial progenitors, which are present in low levels in the circulation. Keratinocytes also start to migrate from the wound edges and proliferate on the surface of the granulation tissue, below the blood clot (Rhett *et al.,* 2008). The base of hair follicles, which is located fairly deep into the dermis, is also an important source of keratinocytes for large area wounds. If these structures are destroyed (as is the case in deep second degree and third degree burns), reepithelialization is very slow and medical interventions, such as skin grafting become necessary.

4.Stage of contraction (maturation and remodeling phase): The duration of the maturation and remodeling phase depends on a number of variables including the patient's genetic makeup, age, location of the wound, type of injury, and duration of inflammation. The remodeling phase envisages replacement of granulation tissue with a frame work of collagen and elastic fibres with revascularization. While the process of granulation has been going in the wound, the epithelium in the periphery has been undergoing hypertrophy and hyperplasia. The epithelium grows to cover the newly formed granulation tissue but does not cover the wound completely until all the exudates have been removed. With the contraction, vessels are obliterated and devascularization results giving the repair of damaged tissues by formation of scar a white or pale appearance (Guo and DiPietro, 2010). A successful contraction results in a smaller wound that needs to be repaired by scar formation. During the maturation phase, type III collagen (which is prevalent during proliferation) becomes gradually degraded and replaced by type I collagen. Collagen remodeling is necessary for the transition from granulation tissue to scar and it relies on the continued synthesis and degradation of collagen. When the balance between collagen synthesis nd degradation is achieved, wound maturation begins (Stadelmann *et al.,* 1998)

The degradation of collagen in the wound is controlled by several proteolytic enzymes called matrix metalloproinases (which are secreted by macrophages), epidermal cells, endothelial cells, and fibroblasts (Mignatti and Rifkin, 1996). During tissue remodeling, PDGF helps to break down old collagen by up-regulating matrix metalloproinases. Other growth factors hich play a role in the remodeling process is TGF-β and FGF (Clark, 1993). With time, the growth of capillaries stops, blood flow to the area declines and metabolic activity at the wound site decreases (Clark, 1993; Falanga, 1998; Baum and Arpey, 2005), resulting in a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength (Falanga, 1998; O'Kane, 2002). In this last phase, the wound has reepithelialized and the dermis has regained most of its tensile strength, although it is no longer as elastic as normal skin, and may be susceptible to re-opening. The scar will continue to undergo further remodeling over a time scale of months to years (Harvey, 2005).

Figure 3. The process of wound healing:



Clinically, wound healing may be described in the following headings:

i. **Healing by first intension or primary intension or primary union or healing of a clean wound (closed wound healing):** when the wound is surgically incised and edges are smooth and in proper apposition, then this type of healing occurs. It is a simplest type of wound healing and wound heals with minimum scar formation and without any complication in a short time. It is the desired type of healing in all the cases. Within 24-48 hours the fibrocytes, which lie at the margins of clean incision, begin to multiply and within 3-4 days from the time of the injury the fibroblasts migrate into the clot material along the fibrin threads. These fibroblasts are accompanied by macrophages as well as capillary buds which unite to form capillary arches. The macrophages dissolve the clot and remove it. Within 4-5 days the clot will have been replaced by vascular connective tissue growing in an organized manner. This process of organization joins the edges of the wound and replaces the previous fibrinous union by a fibrous union and the wound rapidly gains a tensile strength as the fibroblasts assume the characteristics of the fibrocytes. A high level of the tensile strength is attained within 10 days. Factors essential for healing of wound by first intention: recent wound, arrest of hemorrhage, absence of infection, the lips of the wound must be regular, absence of gaping in the depth of the wound, suturing of the wound edges.

ii. **Healing by second intension:** It is the most common way of healing in veterinary practice. This type of wound healing is seen in wounds having gap and contamination (septic wound). This is characterized by exudation, sloughing, inflammation, formation of granulation tissue and epithelialization. In both the cases, the pathological changes are very similar. Where there is extensive loss or gap, the defect is filled with large quantities of granulation tissue. With minor degree of infection this layer of granulation tissue gradually advances till the defect is filled at the same level of the adjacent tissues. At this stage the growth of this granulation tissue should be arrested and epithelialization at the edges of the wound should be initiated to cover this granulation tissue. Irritation persists, the granulation tissue continues to be produced and elevated mass of redundant connective tissue grows at the site of the wound (over growth or proud flesh). Sometimes a scar continues to grow even after the area has been covered by the epithelium. This mass of proliferating connective tissue is known as “Keloid”. In such wound the healing has to progress from the base upward as well as from the edges inwards. This type of healing is slow and results in a large scar. To minimize scar formation and to accelerate healing time, different wound dressings and different techniques of skin substitution have been introduced in the last decades. Autologous skin grafting in the form of split- or full-thickness skin is stills a criterion standard. However, in many patients, this technique may not be practicable for a variety of reasons, and the wound must be allowed to heal by second intention. Moreover, in cases in which skin grafts are used, a new wound is created on the donor side. Thus, eliminating a new wound to close the old one and to close as many tissue defects as possible without the risk of large area infection, necrosis, tissue hypertrophy, and contraction, as well as deformation of wound borders, is a necessity. The next important problem is to reduce or eliminate scar formation, particularly in the field of large-surface wounds.

iii. Healing by third intension or secondary suture: It is the late approximation of a granulating wound surface. It is a useful surgical procedure for many accidental grossly contaminated wounds. On immediate closure, such wounds become infected, so it is left open for 3-10 days and then closed by suture if there is minimal exudation and healthy looking granulation tissue.

iv. Healing by mixed Intension: In this type of healing, some parts of the wound heal by first intension while other parts heal by second intension. This happens when a sutures wound has partially disrupted.

v. Healing under scab: This type of healing is seen in superficial wounds which are not subjected to repeated injury. This occurs mostly in small wounds and abrasions. The blood and lymph which appear on the surface coagulate and become dry, producing dark brown crust. Appearance of granulation tissue takes place under this crust in an identical manner with the healing by second intension and at the same time the marginal epithelium proliferates to cover this granular surface. The exudates present in the wound dries up and form a scab. Underneath this scab the healing process takes place and when it is complete the scab automatically separates and is cast off.

2.4. **Factors which influencing wound healing:**

Local factors that impair host resistance to infection include foreign bodies, necrotic or ischemic tissue, closure of wound under tension, irradiation, hematoma formation, wound dead space, excessive or inappropriate suture material (Tobin, 1984). Systemic disease impairing host resistance include diabetes mellitus, Cushing's disease, hypoproteinemia, feline leukemia virus, feline infectious peritonitis, T-lymphotrophic lenti virus in cats (Waldron and Trevor, 1993), hypovolemic shock, increasing age and malnutrition (Tobin, 1984), hepatic, renal and cardiac diseases (Swaim, 1980).

Wounds in the young patients with good health and on an adequate plane of nutrition heal rapidly whereas those in older patients heal slowly because of the decreased ability to form granulation tissue and increased susceptibility to infection. Protein deficiencies delay wound healing by reducing the number and activity of fibroblasts in the granulation tissue (Peacock and Van Winkle, 1976; Swaim, 1980). When serum protein level falls below 6.0 g/dl, wound healing is slowed and at less that 2g/dl levels, wound healing is markedly inhibited (Waldren and Zimmerman-Pope, 2003). Hypovolemic anemia alters the vascular dynamic leading to local hypoperfusion and hypoxia (Swaim, 1980). Anaemic patients may suffer from other deficiencies including malnutrition, which can impair wound healing (Harvey *et al.,* 1990). Within limits the reduced blood cells does not appear to be a factor in the reduced rate of wound healing (Peacock and Van Winkle, 1976; Swaim, 1980).

Successful wound healing depends on the timely and optimal functioning of many diverse processes, cell types, molecular mediators, and structural elements (Velnar *et al.,* 2009). The outcome of uncomplicated healing is a fine scar with little fibrosis. If a wound does not heal in an orderly or timely sequence, or if the healing process does not result in structural integrity, then the wound is considered chronic (Stadelmann *et al.,* 1998). Although, it is easy to define a chronic wound, finding a solution is complicated. Non-healing wounds are stuck in a constant inflammatory state because they fail to progress through the normal stages of wound healing (Cherubino *et al.,* 2011). Chronic wounds take a longer time to heal or sometimes even recur due to the underlying pathology. Hence, the underlying problem should first be identified and treated accordingly. When treating chronic wounds, it is important to note that biofilms play an important role in the prevention of wound healing. These biofilms harbour various microorganisms which delay the wound healing process. Due to the rise in antibiotic resistance, alternative/traditional medicines are increasingly becoming popular to overcome these multi- resistant organisms. Patients with chronic wounds require prolonged periods of dressings and this can cause a significant financial burden to the health-care system. With the advent of alternative and traditional wound care products, the financial burden can be significantly reduced. The concept of moist wound healing has been generally well accepted and many practitioners are adopting this method (Lusby *et al.,* 2005). Several traditional wound-care products are currently commercially available incorporating the concept of moist wound healing.

The main purpose of the healing of wound is to restore the injured part fully to its previous condition in a short period of time. Early healing of a wound has been the field of attraction for researcher. Despite tremendous advancement, an effective wound management continues to be a challenge to the clinician. In addition to conventional therapies including antibiotics, analgesics, anti-inflammatory drugs many medicinal plants preparation and biological dressing agents have been used to enhance the rate of wound healing. Mostly the wounds are managed by topical medicament, but action of many such common preparations of often discouraging because of ensuing destruction of leucocytes and other cellular elements of the wound (Tyagi and Singh, 1993).

Classical management of wounds follows various therapeutic steps, starting with an aseptic dressing and ending with the rehabilitation of the normal structure and function (Sushruta, 1989). These therapeutic measures were aimed not only to accelerate the healing process but also to maintain the quality and aesthetics of the healing. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are relatively non-toxic, have fewer side effects and are easily available at affordable price. As described in different literature, 70% of the wound healing drugs are of plant origin, 20% of mineral origin, and the remaining 10% consisting of animal products. (Biswas *et al.,* 2003; Bhattacharjee, 2004).

These drugs are stated to be effective in different conditions such as wounds, ulcers, sinuses, abscess, syphilitic ulcers, and maggots in wounds, septic wounds, and inflammatory changes of wounds, cellulitis, purulative ulcer, diabetic carbuncle, and fistula-in-ano. The plants are used as first aids, washing of wounds, extraction of pus, as coagulants and for infected wounds. Clearly strategic planning for research in herbal medicine is needed. The lack of a pharmacological basis for the efficacy and toxicity and clinical data on the majority of herbal medicines is the major constraint to the integration of herbal medicine into conventional medicinal practices (Singh *et al.*, 2005).

2.5. *Salix* treatment of wounds:

Salix spps. is distributed throughout tropical and sub tropical parts of India, Sumathra and Java (Kirtikar and Basu, 1975). Species of *Salix* found in almost all regions of Kashmir as *Salix acmophylla*. Willow and "bains/ Wir/ Veer Kani" respectively, are common English and vernacular names for a number of sister trees of the genera Salix representative of family Salicaceae. These are fast growing and yet medium-sized deciduous trees. It grows primarily in the cool, fertile, irrigated lands as it requires larger quantities of water, though it can withstand cold winter frost. (Rather *et al.*, 2010). They are of enormous ecological and economic importance Argus (1997). As per the Unani system of medicine the leaves of willows give "cold dry" effect while the flowers display "cold wet" effect. It is an ornamental plant, has been reported to possess strong anti-inflammatory property and is used as astringent antiseptic, eye tonic, antipyretic, analgesic and cardiotonic in Indian system of Medicine (Kallman, 1994; Chopra *et al.,* 1996; Bhattcharhjee, 1998).

The active constituents in *Salix* species are: Glycosides (1.5-11%) (Bissett, 1994; Mc Guffin *et al.,* 1997), salicylates (salicin, salicortin, populin, fragilin, tremulacin) (Meier *et al.,* 1985; Zaugg *et al.*, 1997); Tannins (8-20%) (Thieme, 1968); Aromatic aldehydes and acids: salidroside, vanillin, syringin, salicylic acid, caffeic and ferulic acids; Salicyl alcohol (saligenin);flavonoids, etc (Bissett, 1994; Mc Guffin *et al.*, 1997).

Plants play a vital role in maintaining human health and contribute towards the improvement of human life. They are important components of medicines, cosmetics, dyes, and beverages etc. (Khare, 2004). Although hundreds of plant species were tested for antimicrobial properties (Nair and Burke, 1990). There are many cases of infection by drug resistant bacteria whereas few drugs are available effective for the treatment of such patients. Thus, it is urgently necessary to discover or develop new drugs that are effective on such drug resistant bacteria. We have been trying to discover novel compounds, such as antimicrobial compounds and inhibitors of drug resistance systems in bacteria, (Horiuchi *et al.,* 2007) that are effective AGAINST MULTIDRUG-RESISTANT bacteria. Though *salix* is known as one of the herbs that has antimicrobial activity, there are few papers that have shown their antibacterial activity, and have shown anti-fungal, antiviral properties that make it a useful weapon in combating many illnesses (Ali and Aboud, 2010).

Gross and Greenberg (1948) stated the history of aspirin and the present day salicylates has its origins in the use of various salicylate- containing plant extracts. About 2400 years ago, Hippocrates recommended juices of the poplar tree and willow bark for the treatment of eye diseases and pain in childbirth, respectively In the monumental Papyrus Ebers (circa 1550 BC), it is stated that a remedy to expel rheumatic pains (phlegma) in the womb is the application of dried leaves of myrtle, which contain appreciable salicylates. Thus, the analgesic and anti-inflammatory properties of plant extracts containing salicylates have been recognized from these early times. He stated that a boiled vinegar extract of willow leaves could be employed for the relief of pain owing to prolapse of the uterus and other conditions, a paste made from the ash of willow bark for removing corns and callosities and a boiled aqueous extract of willow leaves or ash of willow bark could be used for treating corns, skin diseases, gout and earache. In the second century AD, Galen employed the antiseptic properties of willow leaves (which are now known to contain salicylates) for the treatment of various skin conditions such as wounds, ulcers and erysipelas. Thus by Roman times there were numerous different therapeutic applications for salicylate-containing plants, many of which are appropriate by today's standards. Salicyl alcohol (or saligenin) and its glycoside (salicin) occur in the willow and poplar trees. Many of these naturally occurring salicylates (including salicylic acid itself) are therapeutically effective as weak anti-inflammatory, analgesic and/or antipyretic agents; although in some instances their potency is less than that of aspirin.

Moore (1979) reported that *salix* extract is strong and being antiseptic can be used as a poultice or a wash. He also reported that when used as an analgesic, willow treats urethra, bladder irritation, infected wounds and eczema.

Hart and Jafferey (1981) reported that a poultice of *salix* is applied to bleeding cuts for hemostasis and healing.

Malterud *et al.* (1985) reported that flavonoids present in *salix* to have antifungal properties.

Ho *et al.* (1985) reported that in China, preparations of the bark of the poplar tree (*Populus alba* L.) and decoctions of young shoots from *Salix babylonica* L. have been used for centuries for treatment of rheumatic fever, colds, haemorrhages and goitre, and as a general antiseptic for wounds and abscesses.

Ahmed *et al.* (1995) examined effects of *Matrucaria chamomilla* lotion and ointment, *Salix fragalis* lotion, *M. Chamomilla* lotion and *Polygonum bistorta* ointment, *S. fragalis* lotion and *P. bistorta* ointment, *Nigella sativa* lotion. They observed on the basis of clinical, pathological, histochemical and microbial studies that the healing was best with *M. Chamomilla* lotion followed by *M. Chamomilla* lotion and *Polygonum bistorta* ointment, *Nigella sativa* lotion, *S. fragalis* lotion and *P. bistorta* ointment and with *S. fragalis* lotion the least.

Pojar and Mackinnon (1994) recorded *Salix spps*. (willows) are the source of the natural precursor to asprin, salicylic acid, found in leaves and bark.

Moerman (1998) stated that the active ingredient of the *salix* bark is called salicin. Salicin hydrolyzes in aqueous media to glucose and salicylic alcohol (saligenin). The bark and leaves can be pounded and applied to wounds as healing agents.

Elias (1998) reported that *salix* bark and leaves are pounded and applied to wounds as healing agent.

Mantani *et al.* (2001) reported that catechin present in *salix* and other plants to have antifungal and antimicrobial properties

Barnes *et al.* (2002) reported that, pharmacologically, the *salix* exhibits anti-inflammatory, anti-rheumatic, antipyretic, antidotic, antigesic, and antiseptic properties.

Raskin (1992) mentioned the reason why salicylates are produced in such relatively high abundance by plants is probably because of their roles as growth regulators and in host defence. Principal systems involved in mediating SA-related host defence systems: • Signalling in plant defence against pathogens/injury by local and Systemic Acquired Resistance (SAR), • SAR is mediated by SA-dependent and SA-independent pathways,• Induction of pathogenesis-related resistance systems (PRS). It mechanisms are: • Gene expression of PRS range by SA from early, early to intermediate, or late responses, • Early responses involve transcription factor activation, i.e. signalling via MAP-type and other kinases → phosphorylation/dephosphorylation cycles and (NF B/I B)-type responses, • Increases in ion fluxes (Ca, H /K); modulation by salicyl radical, nitric oxide (NO), H2O2 or lipid peroxides, • Localised cell death → containment of infection, • Late-response genes activated to produce PRs,• Activation of heat shock proteins (HSPs),• Induction of 13-lipoxygenases, • Salicylate metabolised to methyl salicylate, salicyl alcohol and glucoside derivatives -serves to regulate amount of salicylate available for cell actions.

Rainsford (2004) described the importance of the mitochondrial actions of the salicylates in the development of induced cellular death (apoptosis) brings together the observations of the 1950s and 1960s regarding the effects of salicylates on the uncoupling of oxidative phosphorylation and their effects on intermediary metabolism, with more recent data on the activation of caspases and cytochrome- c release from mitochondria. This may be important, along with the newer information on the actions of these drugs on oxyradical and cytotokine-mediated signal transduction events, in understanding the protective effects of aspirin and related drugs in colon and other cancers as well as the mode of action of these drugs in the development of gastrointestinal ulceration and bleeding. As a further example, the long- debated and important therapeutic question regarding the efficacy of high-dose aspirin compared with that of salicylic acid, its dimer, salsalate, or the sodium salt in the long-term treatment of pain and inflammation in rheumatic diseases has been revisited again with evidence that the major anti- inflammatory and analgesic actions of aspirin reside in the salicylate that is produced there from. Current interest in the COX-2 selective NSAIDs highlights important competitive issues for the salicylates-amongst the oldest of the analgesics. Aspirin and the salicylates have faced such competition in the past, including from ibuprofen and paracetamol. However, one single outstanding therapeutic action in the prevention of coronary vascular disease and stroke, which resulted from the discovery of the antiplatelet effects of the drug over three decades ago, has given aspirin a new lease of life. Intense interest in the mode of action of aspirin (which stems from recent understanding of the molecular biology of the cyclo-oxygenases, apoptosis and other components of the regulation of cell cycle and growth) coupled with clinico-epidemiological evidence that it might prevent colon and maybe other cancers now gives further scope for the therapeutic use of aspirin and others of its class.

Saarikoshi *et al.* (2004) stated that *salix* is used as wash for wounds, minor skin inflammations and ulcers.

Ali and Aboud (2010) observed that the highest activity at 100 and 121^{o}C was demonst rated by the methanol extracts of *Salix acmophylla* against *Staphylococcus aureus* and *Klebsiella* spp. While in methanol extracts of *Salvia* *officinalis* the 45°C was the effective temp erature. In this study plants extracts against gram negative bacteria showed activity in acidic p^H only in contrast of gram positive bacteria which were constant in all plants extract. *Salvia officinalis* contained essential elements at higher levels than *Salix acmoplylla*. Ten elements, Ca, Co, Cu, Mn, Fe, K, Na, P, Zn and Pb, were determined in *Salvia officinalis* and *Salix acmophylla*. *Salvia officinalis* contained essential elements (Mn, Fe, K, Na, P and Pb) at higher levels than *Salix acmophylla.* Ca and Zn were present at high levels in *Salix acmophylla* than other. Therefore, it may not produce any health risks for human consumption, if other sources of toxic metal contaminated food are not taken at the same time. The results of this study suggest the possibility of using the methanolic extracts of these plants in treating diseases caused by the test organisms, especially when prepared at acidic pH.

Zarger *et al.* (2014) stated that *salix* spps. have abundant watery bark sap, which is heavily charged with salicylic acid. Besides, salicin, it contains flavonoids and proanthocyanidins, which are potent wound healing agents.

2.6. Herbal treatment of wounds:

China, India, and Egypt appear to have been the places which cradled the use of herbs, but herbalism was common in India. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain an active ingredient, parts of plants, or other plant materials, or combinations. Before the advent of modern medicine, people of all continents have used medicines from plant origins since pre-historic times. Ancient Egyptian medicines of 1000 BC are known to have used garlic, opium, castor oil, coriander, mint, indigo and other herbs for medicine and the Old Testament also mentions herbal use and cultivation, including mandrake, vetch, caraway, wheat, barley and rye. In India, Ayurvedic medicine has used many herbs such as turmeric possibly as early as 1900 BC (Aggarwal *et al.,* 2007).

A great variety of plants are used for medicinal treatments. Either the dried plant, or a specific part of it (root, leaves, fruit, flowers, seeds), these recipes are prepared using different ingredients of non-plant origin such as water, salt, honey, etc. in modern way it is formulated into suitable preparations viz. tablets, pills, extracts, tinctures, lotions, ointments, creams, etc. The first generally accepted use of plants as healing agents was depicted in the cave paintings discovered in the Lascaux caves in France, which have been radiocarbon-dated 13,000 to 25,000 BC (Sushruta, 1989, Bhattacharjee, 2004).

Misra and Agrawal (2006) reported the efficacy of Dermanol® cream (a herbal udder health formulation) on different udder and teat affections in cows and buffaloes studied by various doctors in different states of India and Nepal. They concluded that Dermanol cream had excellent anti-inflammatory, antimicrobial, analgesic and vulnerary actions. Furthermore, Dermanol cream when used as preventive application after each milking not only protected the milch animals against subclinical mastitis and other infective conditions but also kept the teats soft for easy and efficient milking.

Singh and Sahni (2009) found the anti inflammatory and wound-healing properties of *Adhatoda vasica* in 44 cases of wounds in dogs. The wound-healing activity of indigenous plant preparation was compared with a standard herbal drug. The application of 10% ointment of *A. vasica* exhibited significant wound-healing activity. The alcoholic extract exhibited the greatest wound-healing activity (72.33%), followed by the standard ointment (64.38%).

Rizwan *et al.* (2009) reported the traditional use of some important indigenous medicinal plants by the local women in Southern Himalaya Mountains of Pakistan and reported *Bergenia celiata* shrub being used as anti-cancerous drug and curing internal wounds.

Sanyukta *et al.* (2010) reported the quantitative assessment of current status and biomass of *Bergenia celiata* and *Bergenia stracheyi* from Kamaum Himalaya and found that due to difference in climatic condition coupled with wide altitude range provides a wide diversity and reveals the best habitat for obtaining maximum biomass.

Muangman *et al.* (2011) found that the combination of herbal extracts and a silicone derivative in a gel preparation (Cybele® Scagel) have proved effecient on the treatment of deep second degree burns. The application of Cybele® Scagel might have some beneficial effects for prevention of hypertrophic scar formation after partial thickness burn injuries.

Tang *et al.* (2007) recorded the emodin is known to possess wound healing effect, as emodin obtained from rheum species has been found to accelerate the wound healing activity in excisional wound model in rats.

Moslemi *et al.* (2012) treated the wounds with Urtica dioica, and saline solution twice daily and after histological examination concluded that the application of Urtica dioica increased the coetaneous wound healing in cattle.

2.7. Povidone-iodine treatment of wounds:

Kumar *et al.* (2006) irrigated oral surgical wounds with povidone iodine (PVP-1) vs saline and observed cessation of bleeding was achieved in 19 patients treated with PVP-1(76%) and 5 in the saline group (20%). These authors have used a cream and not a wash for treatment of wound in goats.

Vogt *et al.* (2001) used a novel liposome PVP-1 hydrogel complex vs chlorhexidine gauze on meshed skin grafts and reported that treatment with the PVP-1 hydrogel resulted in significantly faster epithelialisation, better antiseptic efficacy, improved wound healing quality and a lower incidence of graft loss in comparison with chlorhexidine gauze.

Miyachi and Imamura (1997) used sugar (70%) and povidone-iodine (3%) paste for a three-year period treating clinical cases of refractory cutaneous ulcers and reported increased granulation tissue formation and a reduction in the size, depth and bacterial contamination of wounds of mixed aetiology.

Konig *et al.* (1997) used PVP-1 and the effect on exotoxins and enzymes in in vitro study and reported that endotoxins and exotoxins released by bacteria have been implicated in delayed wound healing; povidone iodine was found to inactivate bacterial exotoxins such as phospholipase C and lipase and inhibit their further generation. Furthermore, destructive cytokines and enzymes released by neutrophils in response to bacterial colonization were also found to be inactivated.

Pierard-Franchimont *et al.* (1997) used PVP-1 in combination with hydrocolloid dressings vs hydrocolloid dressings alone for four weeks in chronic leg ulcers and reported that PVP-1 accelerated the rate of venous leg ulcer healing when evaluated against hydrocolloid dressings alone. After four weeks the wounds treated with PVP-1 had only a smaller amount of microorganisms present than the hydrocolloid group.

Lammers *et al.* (1990) used 1% povidone-iodine solution to soak the wound before cleaning and debridement vs normal saline and no treatment, on contaminated traumatic wounds (seen within 12hrs of injury) and they took tissue samples before and after soaking and bacterial counts were conducted. While bacterial colonization was reduced with PVP-1 solution, there was no significant difference when compared with the controls.

Knutson *et al.* (1981) used povidone-iodine and granulated sugar mixture to treat patients with wounds, burns and ulcers over 56 month period and reported that this combination rapidly increased the rate of wound healing, reduced the requirement for skin grafting and antibiotics.

Steel (1993) compared efficacy of the most frequently used medications, that is, the Silver sulphadiazine cream, antiseptic solution Povidone-iodine, and 0.9% sodium chloride serum physiologic on the process of healing of the burn injuries and compared them with control group in which no treatment was given. They concluded that there was no significant difference between the treatment groups and control groups.

Mc Lure and Gordan (1992) carried in-vitro study of povidone-iodine (PVP-1) and Chlorhexidine(CHX) against 33 clinical isolates of methicilin resistant Staphylococcus aureus and found that PVP-1 achieved full efficacy with all 33 strains and CHX with only 3 of 33 strains.

Schreier *et al.* (1997) reported good efficacy of Povidone- iodine as antimicrobial agent and also increased tolerability when used along with liposomes.

Lamme *et al.* (1998) carried experiment on full thickness non-infected wounds in pig. They compared topical iodine treatment which consists of a combination of iodine and Cadexomer (modified starch) with Cadexomer ointment, the vehicle without iodine, and with treatment with saline. They found that all 36 wounds healed without wound infection. They also found that wounds treated with Cadexomer-iodine ointment had significantly more epidermal cell layers than other treatments.

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Chapter – 3

MATERIALS AND METHODS

3.1. Place of work:

The experiment was conducted in the Division of Veterinary surgery and Radiology, Faculty of Veterinary Sciences and Animal Husbandry (FVSc & AH), Shuhama, SKUAST-Kashmir- 190006 (J&K).

3.2. Experimental animals:

The experimental study was conducted in eighteen adult rabbits of either sex and weighing 1.8 to 2.5 kg. The animals were purchased from the rabbit section of Mountain Research Centre for Sheep and Goat, FVSc & AH, Shuhama/Wusan rabbitary farm, Pattan. The animals were tagged and housed individually in cages. The animals were provided with commercial diet and water *ad libitum,* maintained under uniform conditions and acclimatized to approaching and handling for a period of 5-10 days prior to the study.

3.3. DESIGN OF EXPERIMENT

3.3. 1. Grouping of animals

The rabbits were randomly divided into 3 groups, of 6 animals each.

3.3. 2. Preparation of the animals

The animals were weighed and subjected to thorough physical and clinical examination before wounding. The wound was prepared for proper aseptic surgical procedures.

3.3.3. Induction of anesthesia

Animals were kept off fed for 8 hours and water was withheld before surgery. Each animal was given xylazine dosed at 10 mg/kg I/M and left in calm environment for five minutes and then administered ketamine hydrocholoride dosed at 50mg/kg I/M (Handoo *et al.*, 2014). Each animal was positioned in sternal recumbency and the dorsal area from the cranial aspect of the thorax to the lumbosacral region was prepared for aseptic surgery.

3.3. 4. Creation of wounds

The location of wound edges was outlined by a locally fabricated metal marker and millimeter ruler (Fig. 4). Four full thickness excisional wounds (1.5 cm ×1.5 cm), 2.5 cm apart from each other were created using a no. 15 BP blade on either side of dorsal spine in the thoraco-lumber region (Fig. 5). The wounds were named as R1 and R2 on right side and L1 and L2 on left side. Haemorrahge, if any, was controlled by digital pressure. Each wound was cleaned with sterile normal saline solution and dressed with as per the scheduled therapy.

3.3. 5. Preparation of *Salix* leaves ointment

The leaves of the plant *Salix* collected were dried and crushed into coarse powder. For topical application 5% w/w ointment was prepared in Vaseline base.

3.3. 6. Therapy of wounds

All the wounds were washed with sterile normal saline solution followed by therapy as indicated below:

Table 1: Therapeutic modalities used in different groups

Group	**No. of animals (No. of wounds)**	**Therapy**
I	6 (24)	No treatment was given (Control)
II	6 (24)	Application of wound with 5% Povidone-iodine (betadin) ointment till healing
III	6 (24)	Application of wound with 5% *Salix* leaves ointment till healing

Each treatment group consisted of 6 animals. Thus each treatment was evaluated on a total of 24 wounds.

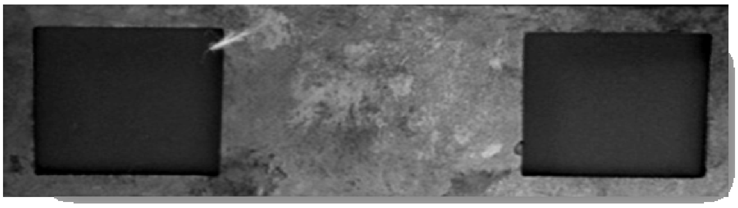

Figure 4: Metal Marker

Figure 5: Nomenclature of the wounds induced on right (R1 and R2) and left (L1 and L2) side

3.4. Evaluation of wound healing:

Parameters to be recorded:

The wound healing efficacy of therapy was evaluated on the basis of clinical observations, wound morphometry, haemato-biochemical, viable bacterial count and histo-morphological studies.

3.4. 1. Physiological parameters:

3.4. 1) Temperature. (^{o}C)

3.4.2) Respiration Rate. (Breaths/min)

3.4.3) Heart Rate. (Beats/min)

were recorded before wounding and then daily up to day 7th and thereafter at weekly interval till day 21st

. **3.4. 2. Gross Examination of wound**:

3.4. 2.1) Appearance of wound

3.4. 2.2) Colour of skin surrounding the wound

3.4.2.3) Degree of Inflammation

3.4.2.4) Appearance of granulation tissue

3.4.2.5) Extent of cicatrisation

3.4.2.6) Presence and type of exudates

Will be evaluated subjectively on day 0, 3rd, 7th, 14th and 21st post wounding

Above parameters were evaluated by using VAS (Visual Analog Score) Card (Handoo, 2013)

3.4.3. Appearance of wound

Wound Edges

0 = indistinct, diffuse, outline not visible

1 = distinct, outline clearly visible, attached with wound base

2 = well, not attached to wound base

3 = well defined, not attached to wound base\rolled under, thickened

4 = well defined, fibrotic, scarred

3.4.4. Colour of skin surrounding the wound

0 = pink or normal

1 = bright red

2 = white or grey pallor

3 = dark red or purple

4 = black or hyper pigmented

3.4.5. Degree of inflammation

0 = clean wound

1 = redness, no swelling, no discharge

2 = redness, swelling, no discharge

3 = redness, swelling, discharge

3.4.6. Appearance of granulation tissue

0 = skin intact, partial skin thickness

1 = bright, beefy red, 75-100% of wound filled

2 = bright, beefy red, 25-75% wound filled

3 = pink or dull red, less or equal to 25% of wound healed

4 = no granulation tissue

3.4.7. Extent of cicatrisation

0 = normal skin colour

1 = fade, thin-out or small white line

2 = light pink, smaller and softened scar

3 = red, larger and thicker areas

4 = scab formation

5 = no scar formation

3.4.8. Presence and type of exudates

1 = none

2 = scant-wound base moist, no measurable exudates

3 = small-wound tissue moist, moisture evenly distributed

4 = moderate-wound base saturated

5 = large-wound tissue bathed in fluid

These exudates will then be classified as

1 = none

2 = bloody thin, bright red

3 = serosangineous-thin, watery, pale red to pink

4 = serous-thin, watery, clear

5 = purulent-thick or thin, opaque tan to yellow

3.5. Wound Morphometry (Handoo, 2013):

3.5.1) **Wound Size:**

The wound boundaries were marked with Indian ink permanent market and tracing was taken on sterile cellophane paper before starting the treatment and subsequently on day 3rd, 7th, 14th and 21st (Fig. 6). These tracings were placed on graph paper and wound area was calculated.

3.5.2) **Percentage Healing:**

The evaluated surface area was used to calculate the percentage healing using the below formula:

$$H = \frac{A - B}{A} \times 100$$

Where,

H	=	Percentage healing.
A	=	Area of wound at the beginning of the experiment.
B	=	Area of wound at the end of particular period.

Percentage healing was calculated on the day of creation and subsequently on day 3rd, 7th, 14th and 21st.

3.6. Hematological parameters

The heparinized blood was collected from ear vein on day 0, 3rd, 7th, 14th and 21st day post wounding. The collected blood was used for hematological, while plasma was used for estimation of biochemical parameters.

3.6.1) Total Erthrocytic Count (TEC) (x 10^6/cu.mm): TEC was calculated manually using the Neubaur's counting chamber using R.B.C. diluting

Fig. 6: Marking the wound edges on cellophane paper

fluid as per the standard technique (Schalm *et al.*, 1975). The result was expressed as million cells per cu mm.

3.6.2) **Total Leukocyte count (**TLC) (x 10^3/cu.mm): TLC was calculated manually using the Neubaur's counting chamber using W.B.C. diluting fluid as per the standard technique (Schalm *et al.*, 1975). The result was expressed as million cells per cu mm.

3.6.3) **Differential Leukocyte Count** (DLC) (%): DLC was estimated using the standard technique (Schalm *et al.*, 1975). The freshly prepared slides stained with Wright-Giemsa stain. The smear were dried and fixed in methanol for one minute and well stained for 30 minutes with Giemsa stain (1:10 dilution). Neutrophils, Lymphocytes, Eosinophils and Monocytes counts were expressed in per cent (%).

3.6.4) **Haemoglobin** (Hb) (gm%): was estimated by using 0.1 N Hcl with the help of Sahli's haemoglobinometer method (Schalm *et al.,* 1975). The values were expressed in g %.

3.6.5) **Packed cell volume** (PCV) (%): PCV was estimated by using microhaematocrit standard technique (Schalm *et al.*, 1975).

3.7. Biochemical parameters:

3.7.1) Total Protein and Albumin (g/dl)

3.7.2) Glucose (mg/dl)

3.7.3) Blood Urea Nitrogen (mmol/L)

3.7.4) Creatinine (μmol/L)

All these parameters were estimated using standard kits (Semi-automated analyser).

3.8. Bacterial Colony Count

For total bacterial viable count, each wound on day of creation and then at day 3^{rd}, 7^{th}, 14^{th} and 21^{st} were washed with 3ml of sterile normal saline solution and washing was collected in sterile test tubes. One ml of each washing was used for serial dilution in NSS for estimating total bacterial count.

Fig: 7: Collection technique of washing solution for bacterial viable count

One ml of each dilution was spread on nutrient agar plates in duplicate. Agar plates were then incubated for 24 hrs at 37^oC. Bacterial colonies from required plates were counted and the total viable count will be calculated (Cowen and Steeel, 1970) as:

TVC=Average no. of colonies in the desired dilution x Dilution Factor

The total viable count will be expressed as cfu/ml of sample.

3.9. Photographic evaluation

Wounds were photographed on day 0, 3, 7, 14 and 21 post- wounding and evaluated by independent observers to assess the quality of wound healing and cosmetic outcome (Arora, 2002).

3.10. Histomorphological examination

The biopsy samples were collected from each group on day 7^{th}, 14^{th} and 21^{st} of wounding and preserved in 10% formalin solution followed by routine procedure for histomorphological examination. Paraffin embedding technique was used and 5-6µ thick sections were cut and stained using H & E stain (Luna, 1968) and Mason's Trichrome stain (Masson, 1929).

3.11. Time required for wound healing

It was recorded on the day on which wound healed completely.

3.12. Statistical Analysis

All results were expressed as Mean ± standard error. The data was analyzed using the suitable statistical program for Social analysis 16 for Windows software (SPSS Inc, Chicago, IL) (Snecador and Cochran, 1989). One way Analysis of Variance (ANOVA) and multiple Duncan range tests were used to compare the means at different time intervals among different group. A value of $P<0.05$ was considered significant.

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Chapter -4

EXPERIMENTAL FINDINGS

In present study, four full thickness excisional skin wounds (1.5 cm ×1.5 cm), 2.5 cm apart from each other were created (dorsal spine of thoraco-lumbar region) on each of 18 rabbits of either sex and weighing 1.8 to 2.5 kg under standard anesthetic protocol to evaluate the wound healing potential of *salix acmophylla* leaves ointment. The total number of wounds evaluated in the study was 72 and thus each treatment groups was evaluated on 24 wounds. Different parameters were recorded and analyzed to compare the healing potential of *salix acmophylla* leaves ointment with Povidone iodine (Bedatin) and sterile normal saline (control).

The results obtained in the present work are described under various headings and sub-headings as follows:

4.1. Effect of treatments on animals:

4.1.1. **Physiological analysis**

4.1.1.1. Temperature (^{0}C)

4.1.1.2. Heart rate (beats/minute)

4.1.1.3. Respiration rate (breaths/minute)

4.1.2. Haematological analysis

4.1.2.1. Total erythrocytic count (x10^6 /cu.mm)

4.1.2.2. Total Leukocytic count (x10^3 /cu.mm)

4.1.2.3. Neutrophils (%)

4.1.2.4. Lymphocytes (%)

4.1.2.5. Mococytes (%)

4.1.2.6. Basophils (%)

4.1.2.7. Eosinophils (%)

4.1.2.8. Haemoglobin (gm%)

4.1.2.9. Packed cell volume

4.1.3. **Biochemical analysis**

4.1.3.1. Glucose (mg/dl)

4.1.3.2. Total Protein (g/dl)

4.1.3.3. Albumin (g/dl)

4.1.3.4. Blood Urea Nitrogen (mmol/L)

4.1.3.5. Creatinine (μmol/L)

4.2. **Evaluation of wound**

4.2.1. Clinical evaluation of wound

4.2.1.1. Percent Score of wound edges (Appearance of wound)

4.2.1.2. Percent Score of Colour of skin

4.2.1.3. Per cent score of Degree of Inflammation

4.2.1.4. Percent Score of Extent of Granulation Tissue

4.2.1.5. Percent Score of Extent of Cicatrization

4.2.1.6. Per cent score of Presence of Exudate

4.2.2. **Morphometry of wound**

4.2.2.1. Wound size

4.2.2.2. Percentage healing

4.3**. Total Bacterial count (cfu/ml)**

4.4. Photographic evaluation

4.5. Histo-morphological evaluation

4.6. Time required for wound healing

4.1. Effects of treatments on animals

4.1.1 Physiological analysis

4.1.1.1. Temperature:

The Mean±SE values of temperature (^{0}C) before and after therapy in animals of different groups have been shown in table - 2 and figure 7.

The value of temperature (^{0}C) in the all the groups were within the normal range without showing any significant difference among them.

However the highest values were recorded in the animals of group I as compared to groups II and III post wounding.

The temperature (^{0}C) showed a non-significant increase from day 1 post wounding in all animals of all the groups except significant increase on day 3 and 5 in group II and significant decrease on day 3 and 5 in group III as compared to base value.

4.1.1.2. Heart rate (beats/minute)

The mean±SE values of heart rate HR (beats/min) before and after therapy in animals of different groups has been shown in table 3 and fig. 8.

The value of heart rate HR (/min) in the all the groups were within the normal range without showing any significant difference among them. However the highest values were recorded in the animals of group II and III.

The HR (/min) showed a significant (P<0.05) increase on day 2, 7 and 14 post wounding in group I as compared to base value. The HR (/min.) showed a significant (P<0.05) increase from day 1 in group II as compared to base value. The HR (/min.) showed a significant (P<0.05) increase from day 2 in group III as compared to base value.

4.1.1.3. Respiration Rate (breaths/min)

The mean±SE values of respiration rate, RR (breaths/min) before and after therapy in animals of different groups have been shown in table 4 and Fig. 9.

The value of respiration rate, RR (breaths/min) in the all the groups were within the normal range without showing any **SIGNIFICANT (P>0.05),** difference in all groups as compared to base value and at different intervals. However the highest values were recorded in the animals of group II and III.

TABLE 2 : MEAN± S.E. VALUE OF DIFFERENT THERAPY ON TEMPERATURE (^{0}C) IN RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

Gp	Observation Intervals (Days)									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21**
I	38.95 ± 0.67^{aA}	39.71 ± 0.42^{aA}	39.76 ± 0.84^{aA}	39.76 ± 0.44^{aA}	39.73 ± 0.44^{aA}	39.46 ± 0.43^{aA}	39.21 ± 0.57^{aA}	39.13 ± 0.56^{aA}	39.01 ± 0.49^{aA}	38.93 ± 0.33^{aA}
II	38.93 ± 0.62^{aA}	39.56 ± 0.51^{aA}	39.40 ± 0.67^{aA}	39.40 ± 0.50^{abA}	39.53 ± 0.48^{aA}	38.98 ± 0.54^{abA}	38.95 ± 0.51^{aA}	38.93 ± 0.41^{aA}	38.78 ± 0.35^{aA}	38.86 ± 0.66^{aA}
III	38.93 ± 0.75^{aA}	39.11 ± 0.77^{aA}	38.78 ± 0.74^{aA}	38.78 ± 0.74^{bA}	39.08 ± 0.73^{aA}	38.82 ± 0.78^{bA}	38.83 ± 0.69^{aA}	38.65 ± 0.44^{aA}	38.65 ± 0.36^{aA}	38.60 ± 0.55^{aA}

Figures with different superscript (small letters) differ significantly ($P<0.05$) between groups
Figures with different superscript (capital letters) differ significantly ($P<0.05$) between days within the groups
n = 6 animals in each group

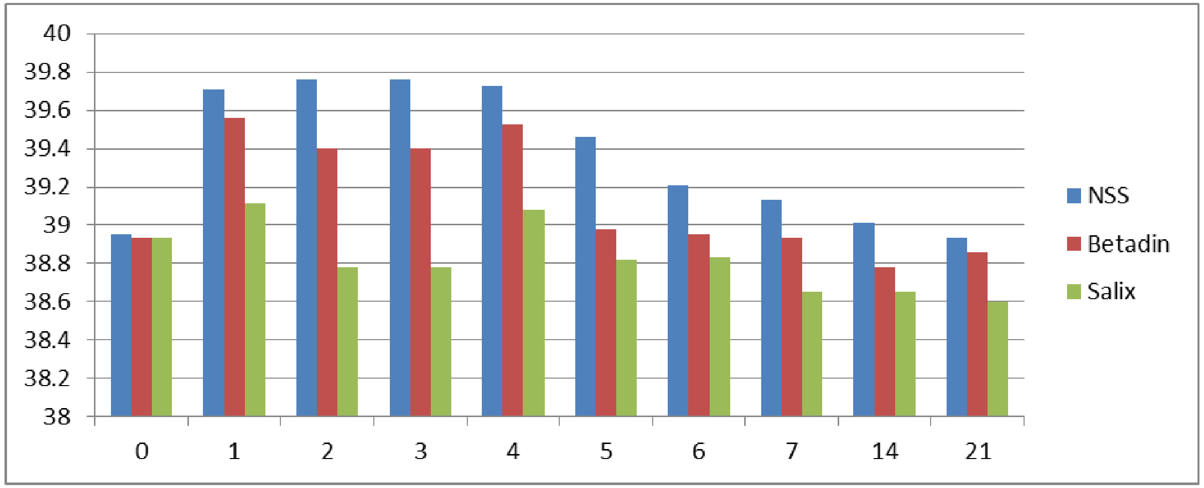


FIG 7: EFFECT OF DIFFERENT THERAPY ON TEMPERATURE (^{0}C) IN RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

Table 3: Mean± S.E. value of different therapy on Heart rate (beats/minute) in rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)									
	0	1	2	3	4	5	6	7	14	21
I	186.00 ±5.36 ^{aA}	191.66 ±6.37 ^{aA}	261.66 ±27.14 ^{aC}	236.6667 ±5.16 ^{aA}	196.33 ±3.66 ^{aA}	236.66 ±5.46 ^{aA}	207.66 ±15.20 ^{aA}	250.00 ±23.66 ^{aBC}	240.00 ±40.49 ^{aBC}	236.00 ±4.73 ^{aA}
II	188.00 ±7.89 ^{aA}	212.66 ±27.03 ^{aAB}	261.66 ±27.14 ^{aC}	250.00 ±23.66 ^{aC}	210.66 ±34.21 ^{aAB}	236.66 ±5.46 ^{aBC}	226.00 ±38.34 ^{aBC}	256.66 ±25.81 ^{aC}	253.33 ±41.79 ^{aC}	249.33 ±20.02 ^{aC}
III	191.66 ±14.10 ^{aA}	216.00 ±34.29 ^{aA}	261.66 ±27.14 ^{aD}	258.33 ±20.41 ^{aCD}	220.66 ±38.91 ^{aAB}	240.00 ±11.09 ^{aBCD}	226.00 ±38.34 ^{aBC}	261.66 ±18.34 ^{aAD}	256.66 ±39.32 ^{aCD}	259.33 ±24.35 ^{aCD}

Figures with different superscript (capital letters) differ significantly ($P < 0.05$) between days within the groups

Figures with different superscript (small letters) differ significantly ($P < 0.05$) between groups

n = 6 animals in each group

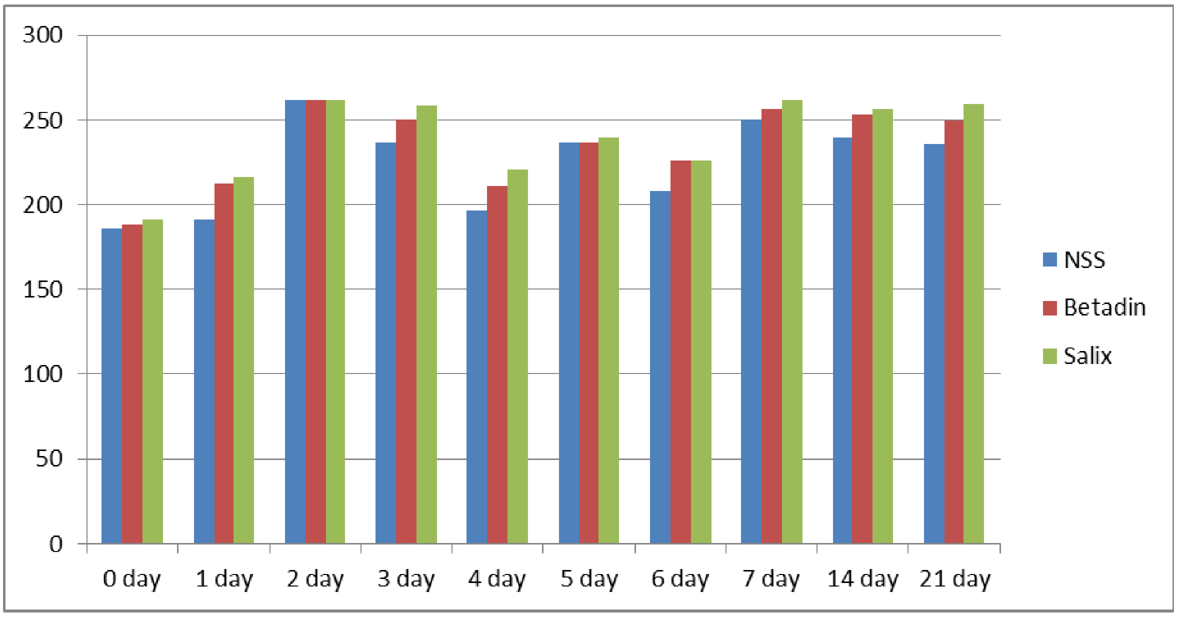


Fig. 8: Effect of different therapy on Heart rate (beats/minute) **in rabbits of different groups at different observation intervals**

Table 4: Mean±SE values of respiration rate (breaths/min) on different therapy in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)									
	0	1	2	3	4	5	6	7	14	21
1	52.66 ±8.54^{AA}	62.33 ±12.80^{AA}	55.66 ±11.48^{AA}	47.00 ±13.60^{AA}	50.33 ±9.99^{AA}	52.00 ±10.73^{AA}	55.33 ±10.48^{AA}	52.00 ±12.13^{AA}	55.33 ±10.48^{AA}	48.66 ±12.24^{AA}
2	52.66 ±8.54^{AAB}	55.66 ±13.41^{AAB}	49.00 ±12.04^{AAB}	48.66 ±12.24^{AAB}	50.33 ±9.99^{AAB}	53.66 ±9.15^{AAB}	52.00 ±7.69^{AAB}	52.00 ±12.13^{AAB}	52.00 ±11.79^{AAB}	40.33 ±7.73^{AAB}
3	45.33 ±8.64^{AA}	50.66 ±14.06^{AA}	44.00 ±5.21^{AA}	47.00 ±13.60^{AA}	47.00 ±9.44^{AA}	47.00 ±9.44^{AA}	52.00 ±7.69^{AA}	48.66 ±12.24^{AA}	48.66 ±11.91^{AA}	39.66 ±4.27^{AA}

Figures with different superscript (capital letters) differ significantly ($P < 0.05$) between days within the groups
Figures with different superscript (small letters) differ significantly ($P < 0.05$) between groups
n = 6 animals in each group

4.1.2.1. Total erythrocytic count (x10^6 /cu.mm)

The mean±SE values of Total Erythrocyte Count (x10^6) before and after therapy have been shown in table 5 and fig. 10.

The decrease in Total Erythrocyte Count was significant (P<0.05) on day 3 and 7 in group I, on day 3, 7 and 14 in group II and on day 7, 14 and 21 in group III as compared to base value. The increase in Total Erythrocyte Count was significant (P<0.05) on day 21 in groups II and III as compared to base value. Total Erythrocyte Count did not show any **significant (P>0.05),** change in all groups at different intervals.

4.1.2.2. Total Leucocytes count (x10^6 /cu.mm)

The mean±SE values of Total Leucocytes count (x10^3) before and after therapy have been shown in table 6 and fig. 11.

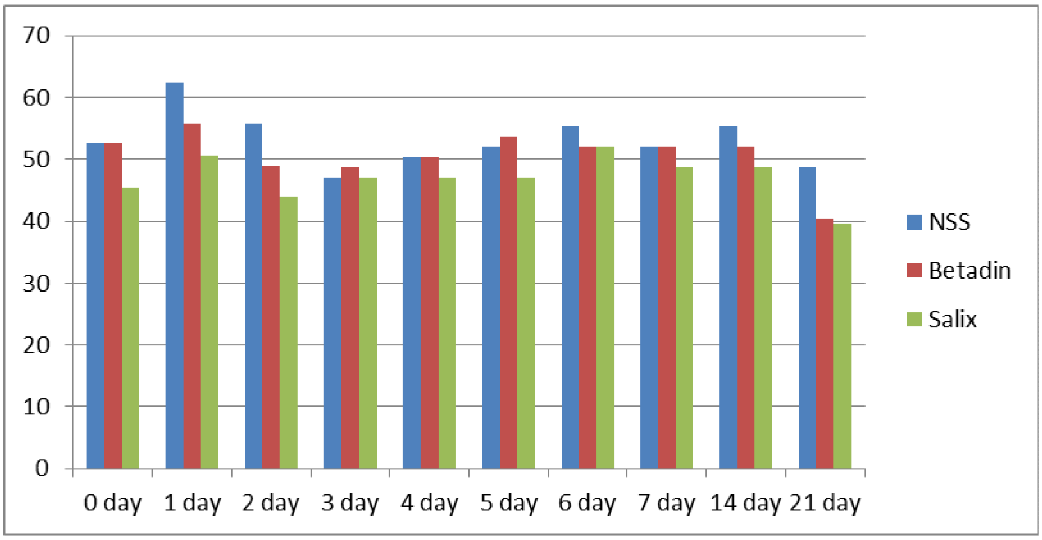


FIG: 9: EFFECT OF DIFFERENT THERAPY ON RESPIRATION RATE (BREATHS/MIN) IN THE RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

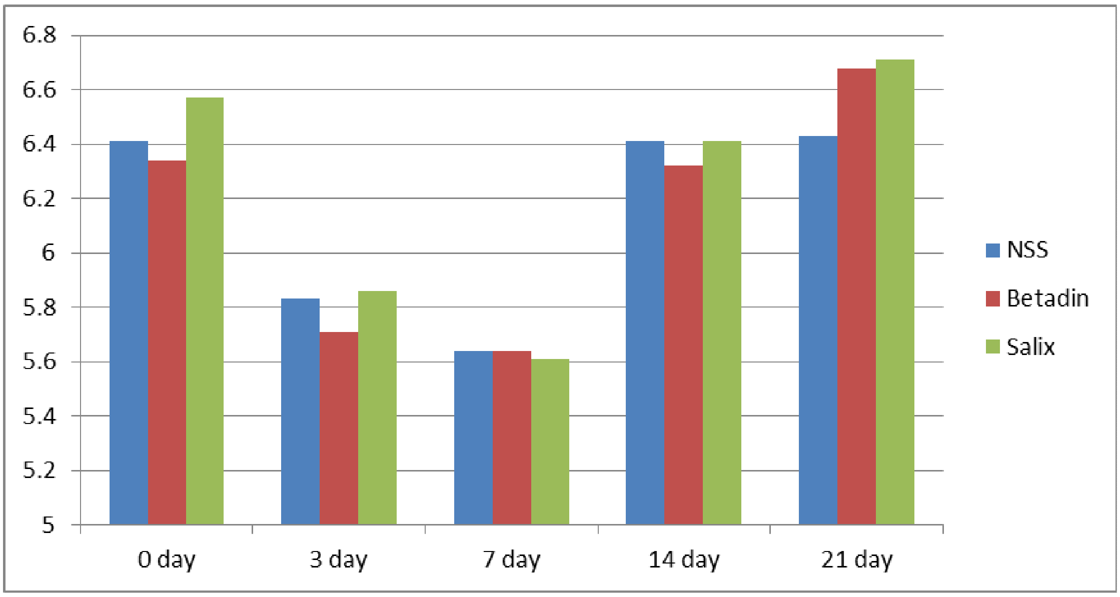


Fig 10: Effect of different therapy on Total Erythrocyte Count (x10^6) in the rabbits of different groups at different observation intervals

A significant (P<0.05) increase in Total Leucocytes count (TLC) was noticed from day 3 to 14 in group I as compared to base value. A significant (P<0.05) increase in TLC was noticed on day 3 to 7 and significant (P<0.05) decrease on day 14 and 21 in group II as compared to base value. A significant (P<0.05) increase in TLC was noticed from day 3 to 14 and significant (P<0.05) decrease on day 21 in group III as compared to base value. In comparison to group I, higher values of TLC were recorded in groups II and III, which were significant from day 3 to 21 higher in group III.

Table-5: Mean±S.E. of Total Erythrocyte Count ($x10^6$) in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	3	7	14	21
I	$6.41±0.21^{aB}$	$5.83±0.33^{aA}$	$5.64±0.30^{aA}$	$6.41±0.31^{aB}$	$6.43±0.19^{aB}$
II	$6.34±0.27^{aB}$	$5.71±0.19^{aA}$	$5.64±0.30^{aA}$	$6.32±0.25^{aB}$	$6.68±0.20^{aC}$
III	$6.57±0.05^{aB}$	$5.86±0.18^{aB}$	$5.61±0.32^{aA}$	$6.41±0.31^{aC}$	$6.71±0.17^{aC}$

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

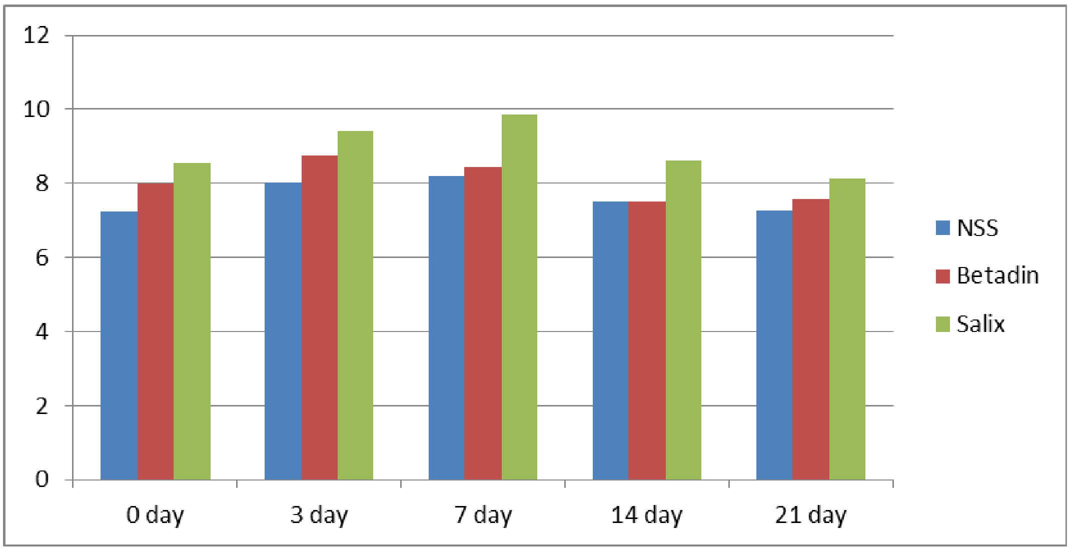


Fig: 11:Effect of different therapy on Total Leucocytes count ($x10^3$) in the rabbits of different groups at different observation intervals

Table-6: Mean±SE of Total Leucocytes count ($x10^3$) in the rabbits of different groups at different observation intervals

GP	Observation Intervals (Days)				
	0	3	7	14	21
I	**7.23±0.59^{AA}**	**8.03±0.86^{ABC}**	**8.21±0.47^{AC}**	**7.51±0.29^{AAB}**	**7.26±0.29^{AA}**
II	**7.98±0.27^{AAB}**	**8.75±0.50^{ABC}**	**8.46±0.90^{ABC}**	**7.51±0.29^{AA}**	**7.58±0.19^{AA}**
III	**8.56±0.52^{AAB}**	**9.41±0.40^{BC}**	**9.85±0.12^{BD}**	**8.60±0.16^{BB}**	**8.15±0.40^{BA}**

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.2.3. Neutrophils (%)

The mean±SE values of neutrophil count before and after therapy have been shown in table 7 and fig. 12.

At different intervals, neutrophil count showed an increasing trend in all the groups throughout the period of study except on day 21. The neutrophils increased significantly (P<0.05) from day 3 to 14 in groups I, II and III as compared to base value. On day 3, the neutrophil count was significantly (P<0.05) decrease in group II followed by group III as compared to group I and day 21, the neutrophil count was non-significantly (P>0.05) decrease in group III followed by group II as compared to group I.

Table-7:Mean±SE of neutrophils (%) in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	3	7	14	21
I	35.73±1.76^{AA}	69.76±6.30^{AC}	66.88±5.34^{BC}	47.68±4.41^{AB}	34.98±0.81^{AA}
II	34.90±1.13^{AA}	67.43±4.07^{AD}	63.21±4.55^{ABC}	49.68±4.58^{AB}	33.65±0.72^{AA}
III	36.06±1.48^{AA}	70.43±3.47^{AD}	60.05±5.31^{AC}	48.18±2.73^{AB}	32.31±0.59^{AA}

Figures with different superscript (capital letters) differ significantly ($P < 0.05$) between days within the groups
Figures with different superscript (small letters) differ significantly ($P < 0.05$) between groups
n = 6 animals in each group

4.1.2.4. Lymphocytes (%)

The mean±SE values of lymphocytes before and after therapy have been shown in table 8 and fig. 13.

At different intervals, lymphocytes showed a decreasing trend in all the groups throughout the period of study except on day 21. The decrease in lymphocytes was significant (P<0.05) from day 3 to 14 in all groups except significant (P<0.05) increase in group III, on day 21 in groups III as compared to base value. On day 7 and 21, the value was significantly (P<0.05) higher in group III as compared to groups I and II.

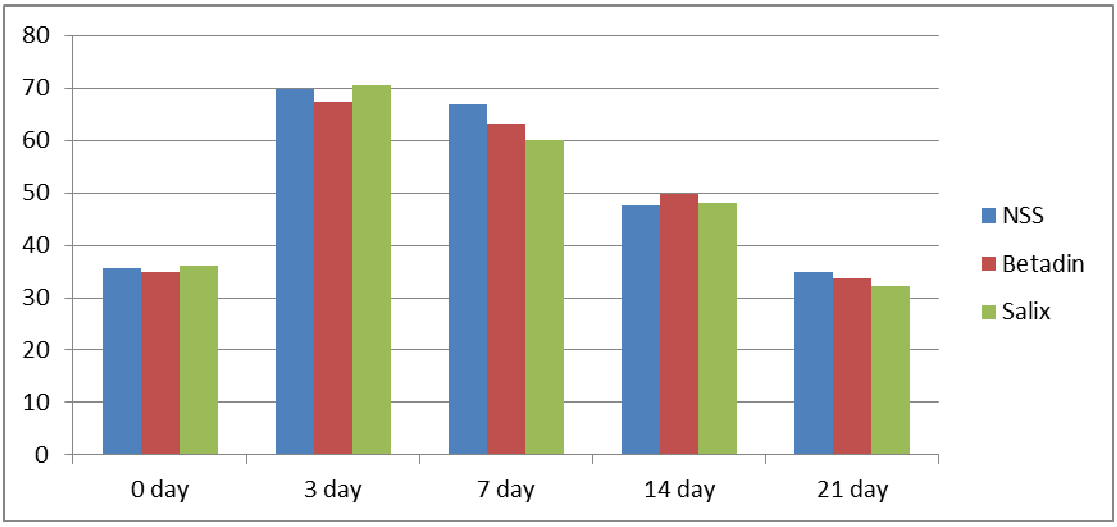


Fig 12: Effect of different therapy on neutrophils (%) in the rabbits of different groups at different observation intervals

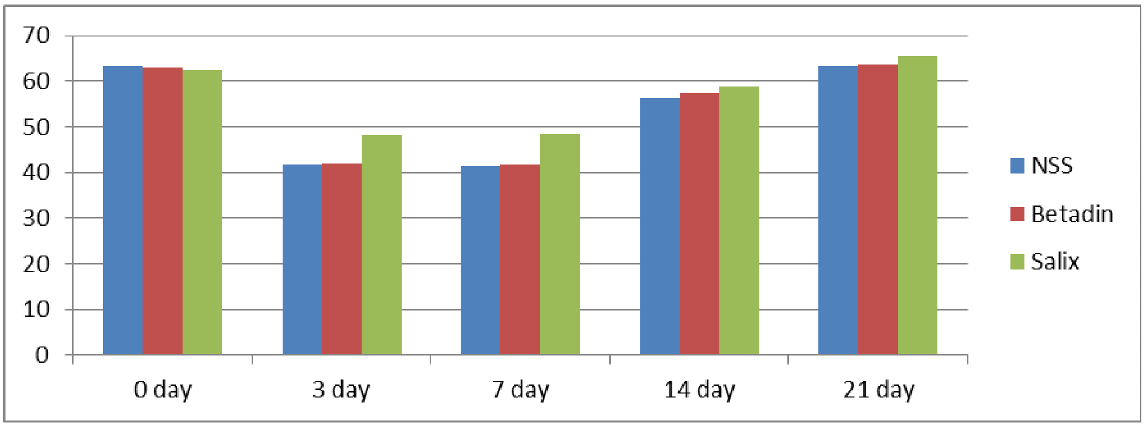


Fig. 13: Effect of different therapy on Lymphocytes (%) in the rabbits of different groups at different observation intervals

Table 8: The mean±SE values of lymphocytes (%) in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	3	7	14	21
I	63.31 ± 0.68^{aC}	41.76 ± 6.65^{aA}	41.55 ± 5.60^{aA}	56.35 ± 1.64^{aB}	63.31 ± 0.84^{aC}
II	63.06 ± 1.37^{aC}	42.10 ± 6.43^{aA}	41.71 ± 5.12^{aA}	57.35 ± 3.87^{aB}	63.48 ± 1.57^{aC}
III	62.40 ± 2.62^{aBC}	48.26 ± 3.81^{aA}	48.38 ± 4.26^{bA}	58.85 ± 3.75^{aB}	65.65 ± 1.84^{bC}

Figures with different superscript (capital letters) differ significantly ($P < 0.05$) between days within the groups
Figures with different superscript (small letters) differ significantly ($P < 0.05$) between groups
n = 6 animals in each group

4.1.2.5. Mococytes (%)

The mean±SE values of monocytes (%) before and after therapy have been shown in table 9 and fig. 14.

The increase in monocytes was significant (P<0.05) on day 3 and then there was significant (P<0.05), decreasing trend in all the groups as compared to base value. Monocytes did not show any significant (P>0.05), change IN ALL GROUPS AT DIFFERENT INTERVALS.

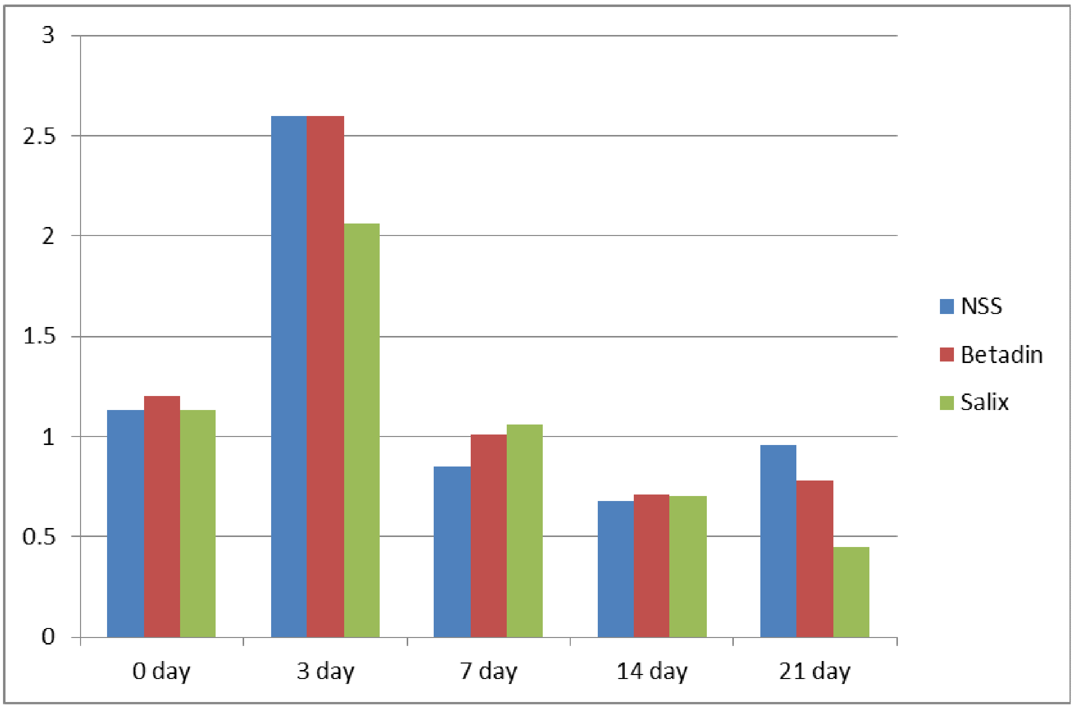


Fig. 14 : Effect of different therapy on Monocytes (%) in the rabbits of different groups at different observation intervals

Table 9: The mean±SE values of monocytes (%) in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	**3**	**7**	**14**	**21**
I	1.13 ± 0.32^{aC}	2.60 ± 0.20^{bD}	0.85 ± 0.20^{aAB}	0.68 ± 0.22^{aA}	0.96 ± 0.05^{aBC}
II	1.20 ± 0.22^{aB}	2.60 ± 0.37^{bC}	1.01 ± 0.38^{aAB}	0.71 ± 0.24^{aC}	0.78 ± 0.24^{aA}
I	1.13 ± 0.21^{aC}	2.06 ± 0.47^{aD}	1.06 ± 0.32^{aBC}	0.70 ± 0.21^{aAB}	0.45 ± 0.23^{aA}

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.2.6. Basophils (%)

The mean±SE values of Basophils (%) before and after therapy have been shown in table 10 and fig. 15.

The value of Basophils (%) in the all the groups were within the normal range without showing any SIGNIFICANT (P>0.05), CHANGE in all groups as compared to base value and at different intervals.

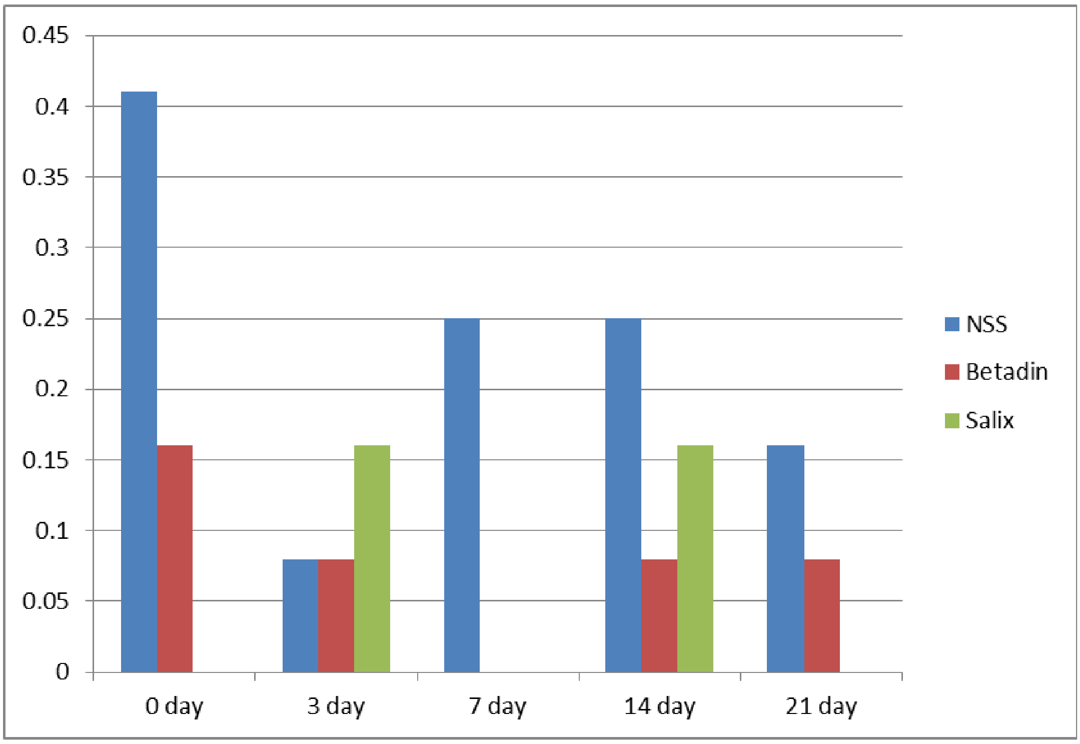


Fig. 15: Effect of different therapy on Basophils (%) in the rabbits of different groups at different observation intervals

Table 10: The mean±SE values of Basophils (%) in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	3	7	14	21
I	0.41 ± 0.37^{aA}	0.08 ± 0.20^{aA}	0.25 ± 0.38^{aA}	0.25 ± 0.41^{aA}	0.16 ± 0.25^{aA}
II	0.16 ± 0.25^{aA}	0.08 ± 0.20^{aA}	0.00 ± 0.00^{aA}	0.08 ± 0.20^{aA}	0.08 ± 0.20^{aA}
III	0.00 ± 0.00^{aA}	0.16 ± 0.25^{aA}	0.00 ± 0.00^{aA}	0.16 ± 0.25^{aA}	0.00 ± 0.00^{aA}

4.1.2.7. Eosinophils (%)

The mean±SE values of eosinophils (%) before and after therapy have been shown in table 11 and fig. 16.

The value of eosinophils (%) in the all the groups were within the normal range without showing any SIGNIFICANT (P>0.05), CHANGE in all groups as compared to base value and at different intervals.

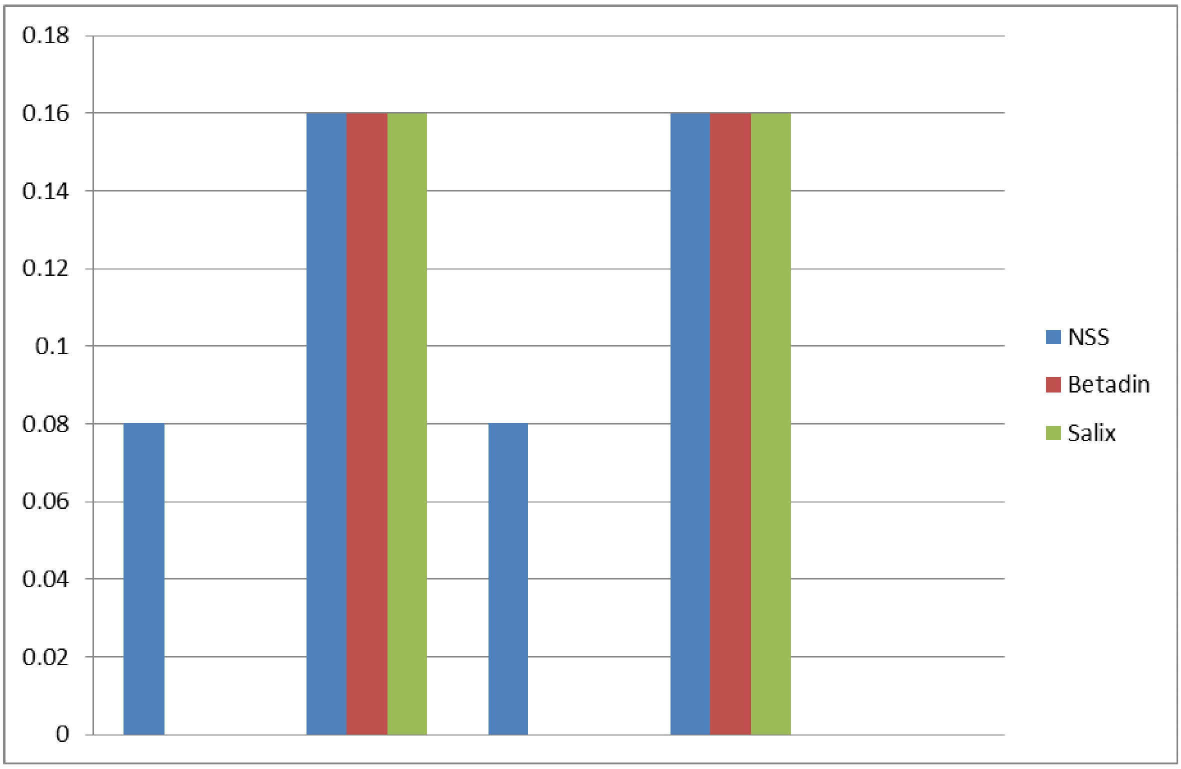


Fig 16 : Effect of different therapy on Eosinophils (%) in the rabbits of different groups at different observation intervals

Table 11: The mean±SE values of eosinophils (%) in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	3	7	14	21
I	0.08 ± 0.20^{aA}	0.16 ± 0.25^{aA}	0.08 ± 0.20^{aA}	0.16 ± 0.25^{aA}	0.00 ± 0.00^{aA}
II	0.00 ± 0.00^{aA}	0.16 ± 0.25^{aA}	0.00 ± 0.00^{aA}	0.16 ± 0.25^{aA}	0.00 ± 0.00^{aA}
III	0.00 ± 0.00^{aA}	0.16 ± 0.25^{aA}	0.00 ± 0.00^{aA}	0.16 ± 0.25^{aB}	0.00 ± 0.00^{aA}

4.1.2.8. Haemoglobin (Hb)

The mean±SE values of haemoglobin (Hb) before and after therapy have been shown in table 12 and fig. 17.

In all the groups, the Hb decreased significantly (P<0.05) on day 3 as compared to base value. In comparison to group I, high levels of Hb was recorded in groups III followed by II and the values were significantly (P<0.05) higher in group group III followed by group II on day 21. In groups I and III, non-significant (P>0.05) decrease on day 7 and day 14 was recorded respectively as compared to base value. In group II, significant (P<0.05) increase value from day 3 to 21 day as compared to base value. However, the value of Hb in the all the groups were within normal physiological limits without showing any SIGNIFICANT (P>0.05), CHANGE in all groups at different intervals.

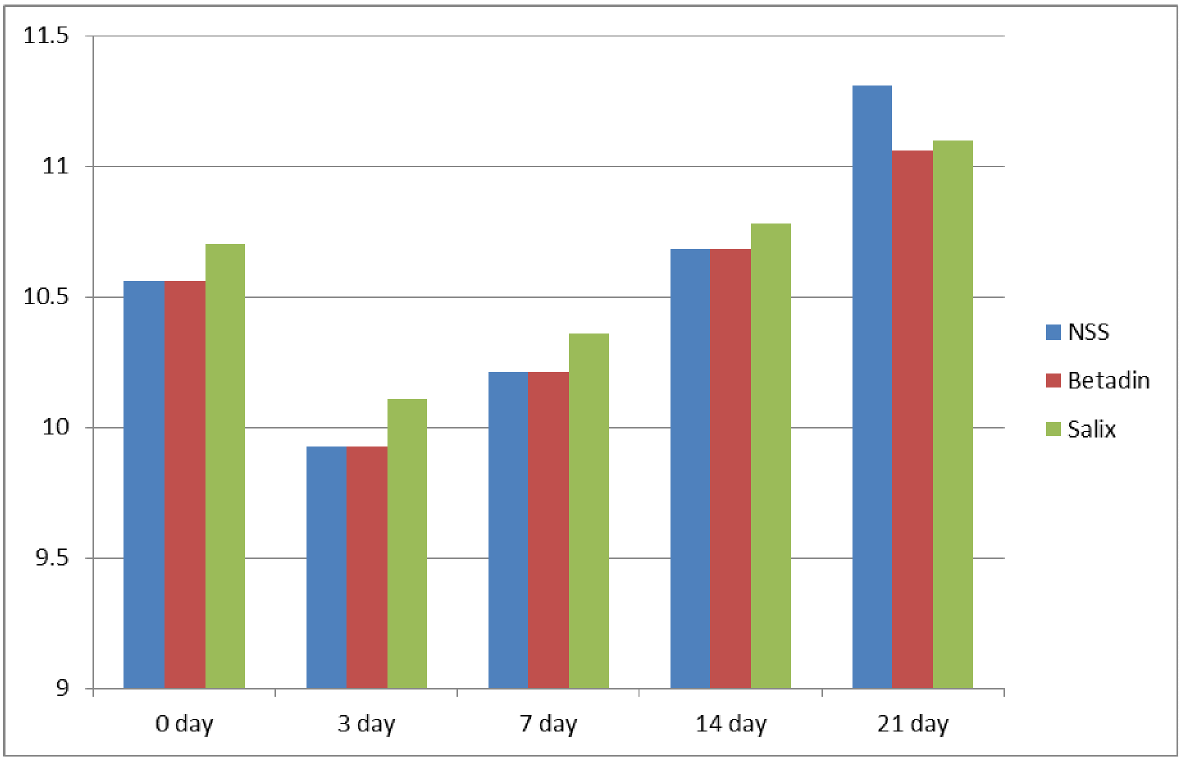


Fig 17: Effect of different therapy on Haemoglobin (g%) in the rabbits of different groups at different observation intervals

Table 12: The mean±SE values of Haemoglobin in the rabbits of different groups at different observation intervals

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	$10.56±0.72^{aAB}$	$9.93±0.35^{aA}$	$10.21±0.37^{aAB}$	$10.68±0.45^{aBC}$	$11.31±0.74^{aC}$
II	$10.56±0724^{aBC}$	$9.93±0.35^{aA}$	$10.21±0.37^{aAB}$	$10.68±0.45^{aBC}$	$11.06±0.46^{aC}$
III	$10.70±0.60^{aBC}$	$10.11±0.34^{aA}$	$10.36±0.34^{aAB}$	$10.78±0.32^{aBC}$	$11.10±0.39^{aC}$

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.2.9. Packed Cell Volume

The mean±SE values of Packed Cell Volume (PCV) before and after therapy have been shown in table 13 and fig. 18.

In all the groups, the Packed Cell Volume (PCV) increased significantly (P<0.05) on day 3 and 7 as compared to base value. However, the value of PCV in the all the groups were within normal physiological limits without showing any SIGNIFICANT (P>0.05), CHANGE in all groups at different intervals.

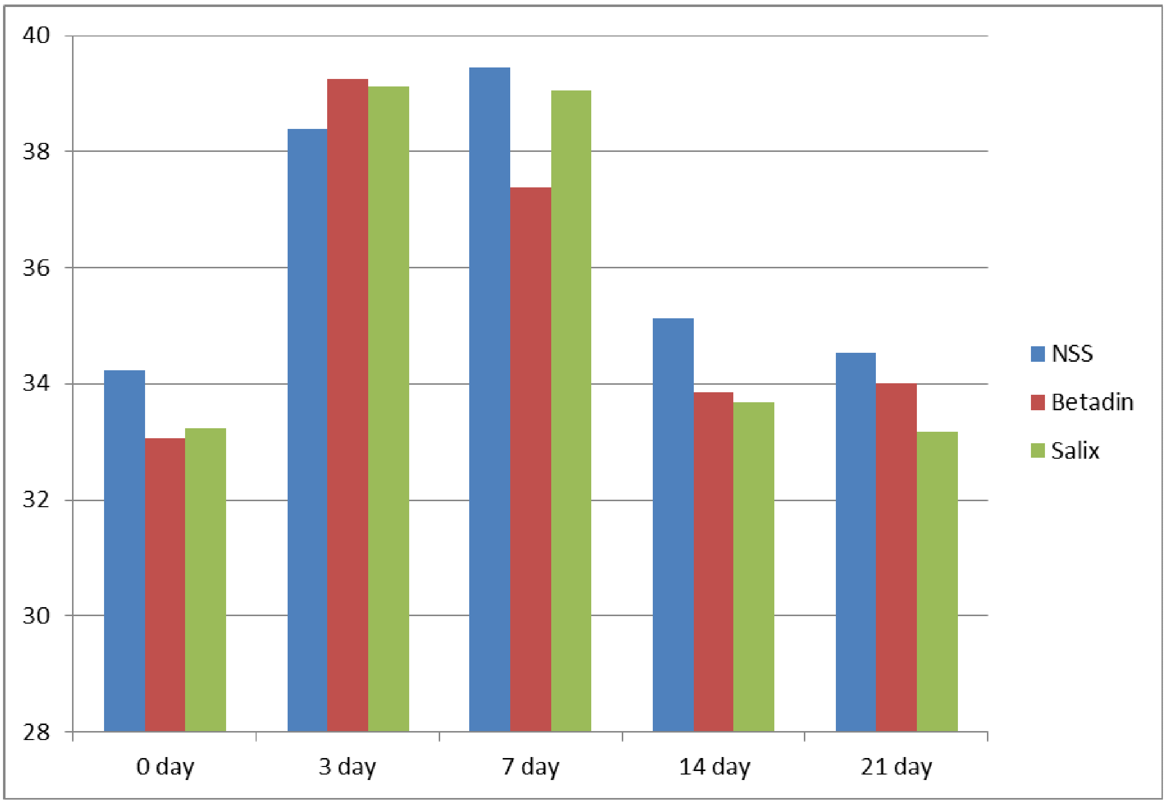


Fig. 18: Effect of different therapy on Packed Cell Volume (%) in the rabbits of different groups at different observation intervals

Table 13: The mean±SE values of Packed Cell Volume in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	**3**	**7**	**14**	**21**
I	34.23 ± 0.93^{aA}	38.40 ± 2.71^{aB}	39.46 ± 1.87^{aB}	35.13 ± 2.60^{aA}	34.53 ± 1.48^{aA}
II	33.06 ± 1.71^{aA}	39.26 ± 0.69^{aB}	37.38 ± 2.75^{aB}	33.85 ± 2.49^{aA}	34.01 ± 0.48^{aA}
III	33.23 ± 1.73^{aA}	39.13 ± 1.09^{aB}	39.05 ± 0.93^{aB}	33.68 ± 1.89^{aA}	33.18 ± 0.77^{aA}

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.3.1. Glucose (mg/dl)

The mean±SE values of glucose before and after therapy have been shown in table 14 and fig. 19.

The glucose showed a non-significant increase (P>0.05), from day 3 to 21 in group I and on day 14 and 21 in group III except on day 3 in group II, where the value was significantly lower (P<0.05) and on day 14 and 21 the value was significantly higher (P<0.05) from the base value. However, the value of glucose

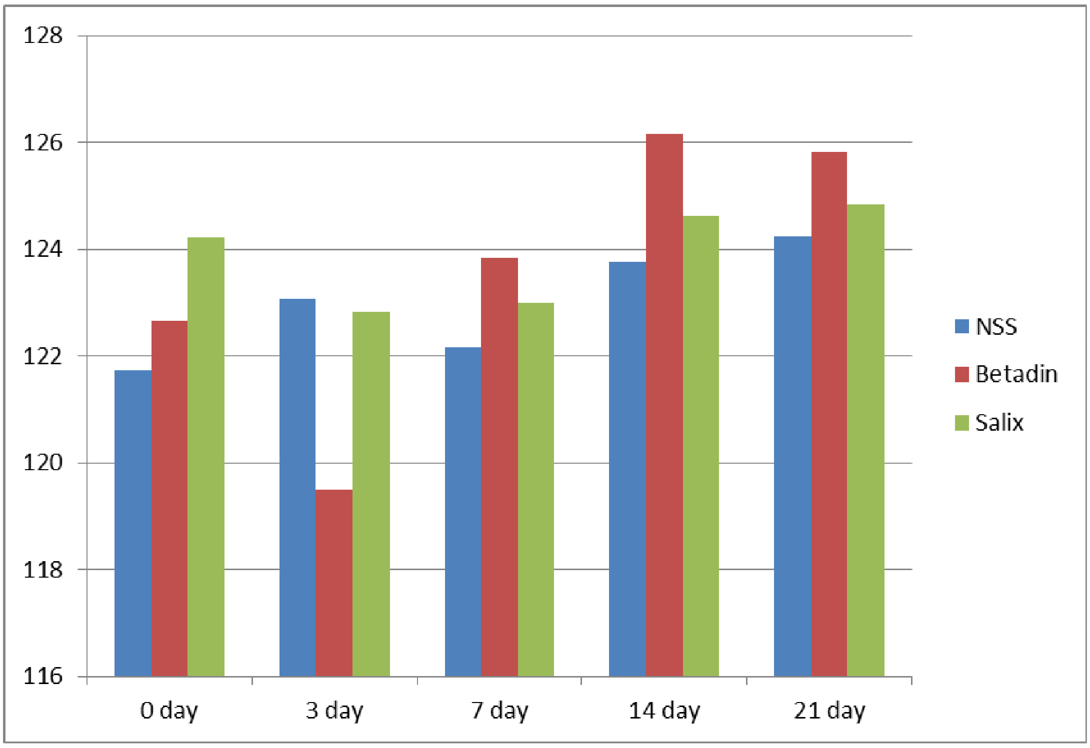


Fig. 19. : Effect of different therapy on Glucose (mg/dl) **in the rabbits of different groups at different observation intervals**

in the all the groups were within normal physiological limits without showing any significant (P>0.05), change in all groups at different intervals.

Table 14: The mean±SE values of glucose in the rabbits of different groups at different observation intervals

Gp	Observation Intervals (Days)				
	0	3	7	14	21
I	121.73 ± 4.48^{AA}	123.08 ± 5.23^{AA}	122.16 ± 4.91^{AA}	123.76 ± 4.84^{AA}	124.25 ± 4.64^{AA}
II	122.66 ± 3.44^{AAB}	119.50 ± 2.16^{AA}	123.83 ± 4.91^{AAB}	126.16 ± 4.44^{AB}	125.83 ± 4.44^{AA}
III	124.23 ± 3.59^{AA}	122.83 ± 2.78^{AA}	123.00 ± 3.79^{AA}	124.63 ± 2.31^{AA}	124.83 ± 1.94^{AA}

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.3.2. Total Protein (g/dl)

The mean±SE values of Total Protein (TP) before and after therapy have been shown in table 15 and fig. 20.

The TP showed a significant decrease (P<0.05) throughout the study period in group I and in group III, where the value was non-significantly lower (P>0.05) and on day 7 and 21 the value was significantly higher (P<0.05) in group II as compared to the base value. However, the value of TP in the all the groups were within normal

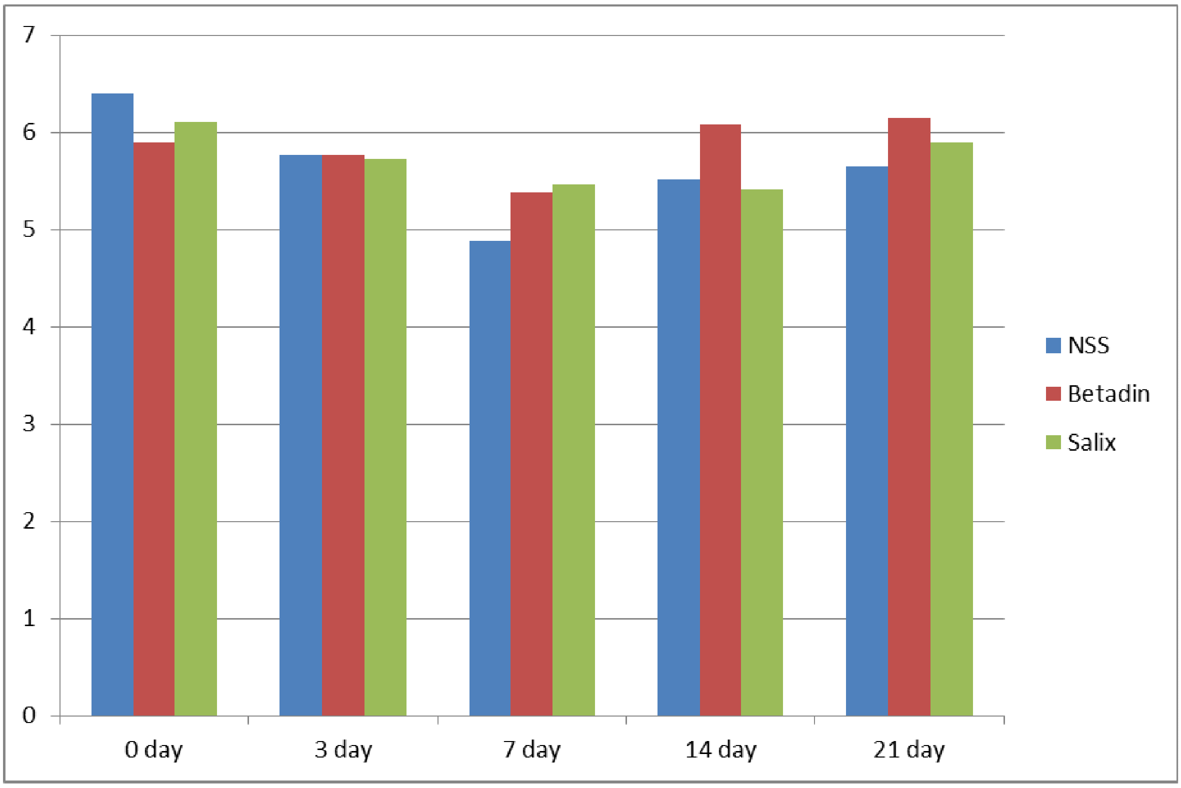


Fig. 20. : Effect of different therapy on Total Protein (g/dl) in the rabbits of different groups at different observation intervals

physiological limits without showing any significant (P>0.05), change in all groups at different intervals.

Table 15: The mean±SE values of Total Protein in the rabbits of different groups at different observation intervals

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	$6.40±1.03^{AC}$	$5.76±0.25^{ABC}$	$4.88±0.54^{AA}$	$5.51±0.29^{AAB}$	$5.65±0.40^{AB}$
II	$5.90±0.43^{AAB}$	$5.76±0.47^{AAB}$	$5.38±0.83^{AA}$	$6.08±0.50^{AAB}$	$6.15±0.53^{AB}$
III	$6.13±0.64^{AA}$	$5.73±0.49^{AA}$	$5.46±0.51^{AA}$	$5.41±0.78^{AA}$	$5.90±0.47^{AA}$

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.3.3. Albumin (g/dl)

The mean±SE values of Albumin before and after therapy have been shown in table 16 and fig. 21.

The Albumin showed a significant decrease (P<0.05), from day 3 to 14 in all the groups except on day 21 in group II, where the value was non-significantly higher (P>0.05) and on day 21 the value was significantly higher (P<0.05) in groups I and III as compared to the base value. However, the value of Albumin in the all the

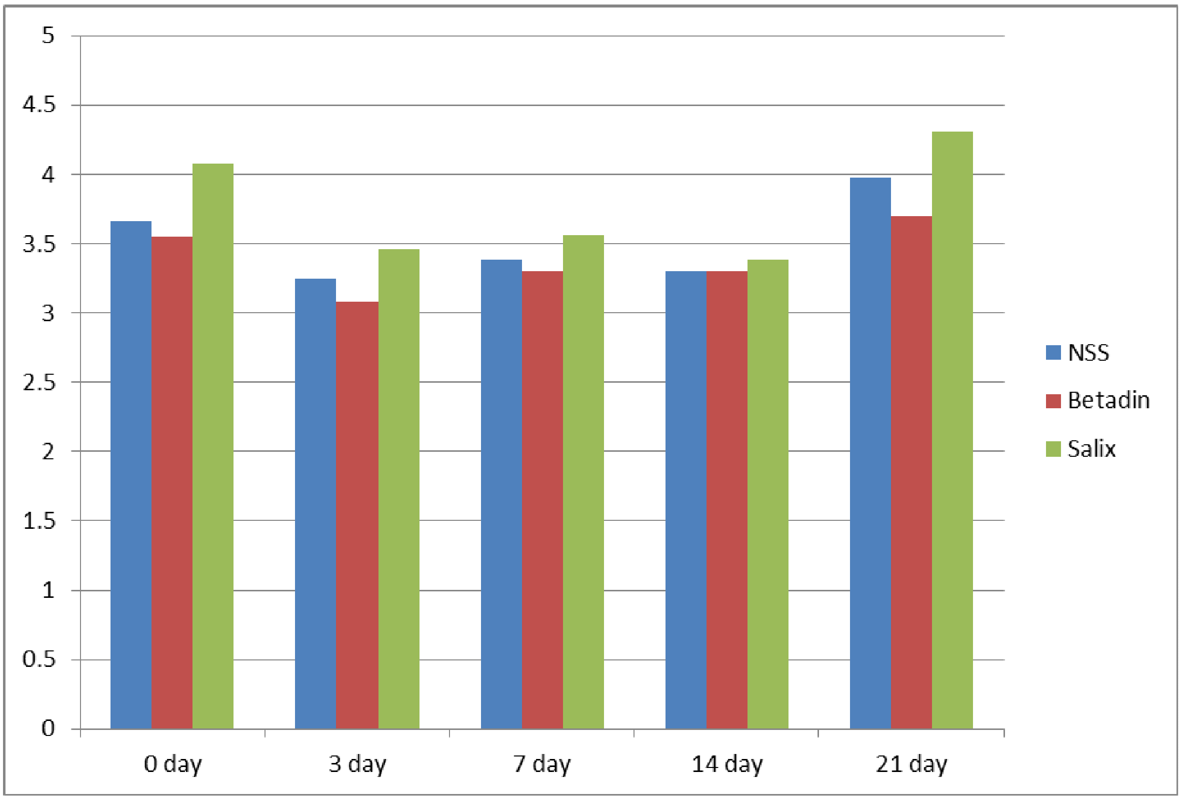


Fig 21: Effect of different therapy on Albumin (g/dl) **in the rabbits of different groups at different observation intervals**

groups were within normal physiological limits without showing any significant (P>0.05), change in all groups at different intervals.

TABLE 16: THE MEAN±SE VALUES OF ALBUMIN IN THE RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

GROUP	OBSERVATION INTERVALS (DAYS)				
	0	3	7	14	21
I	3.66±0.51 AAB	3.25±0.41 AA	3.38±0.49 AA	3.30±0.46 AA	3.98±0.26 AB
II	3.55±0.33 AB	3.08±0.20 AA	3.30±0.40 AAB	3.30±0.46 AAB	3.70±0.30 AB
III	4.08±0.37 ABC	3.46±0.40 AAB	3.56±0.62 AAB	3.38±0.63 AA	4.31±0.47 AC

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.3.4. Blood Urea Nitrogen (mmol/L)

The mean±SE values of Blood Urea Nitrogen (BUN) before and after therapy have been shown in table 17 and fig. 22.

The BUN showed a non-significant decrease (P>0.05) throughout the study period in group I except non-significantly higher (P>0.05) value on day 21 in group I and on day 3 and 7 in group II as compared to the base value. However, the value of

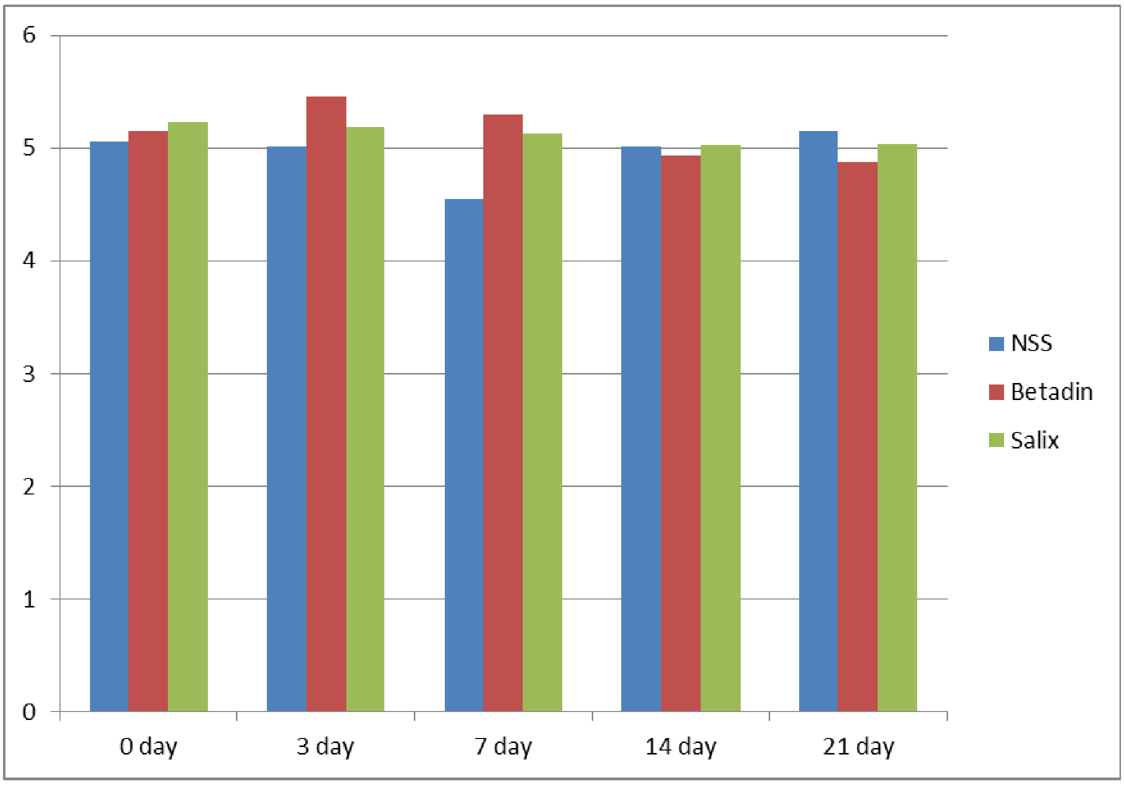


Fig. 22. Effect of different therapy on Blood Urea Nitrogen in the rabbits of different groups at different observation intervals

BUN in the all the groups were within normal physiological limits showing significant (P<0.05) value in II and III groups as compared to group I on day 7.

TABLE 17: THE MEAN±SE VALUES OF BLOOD UREA NITROGEN IN THE RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	$5.06±0.48^{aA}$	$5.01±0.51^{aA}$	$4.55±0.44^{aA}$	$5.01±0.49^{aA}$	$5.15±0.56^{aA}$
II	$5.15±0.77^{aA}$	$5.46±0.53^{aA}$	$5.30±0.37^{bA}$	$4.93±0.51^{aA}$	$4.88±0.60^{aA}$
III	$5.23±0.48^{aA}$	$5.18±0.47^{aA}$	$5.13±0.38^{bA}$	$5.03±0.50^{aA}$	$5.04±0.51^{aA}$

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.1.3.5. Creatinine (μmol/L)

The mean±SE values of Creatinine before and after therapy have been shown in table 18 and fig. 23.

The Creatinine showed a significant increase (P<0.05) from day 3 to 14 except non-significantly higher (P>0.05) value on day 21 in group I and in group II, where the value was significantly higher (P<0.05) on day 3 and 7 and significant (P>0.05) lower value on day 14 and 21 and the value was significantly higher (P<0.05) on day 3 and 7 and significantly lower (P<0.05) in group III as compared to the base value. However, the value of Creatinine in the all the groups were within

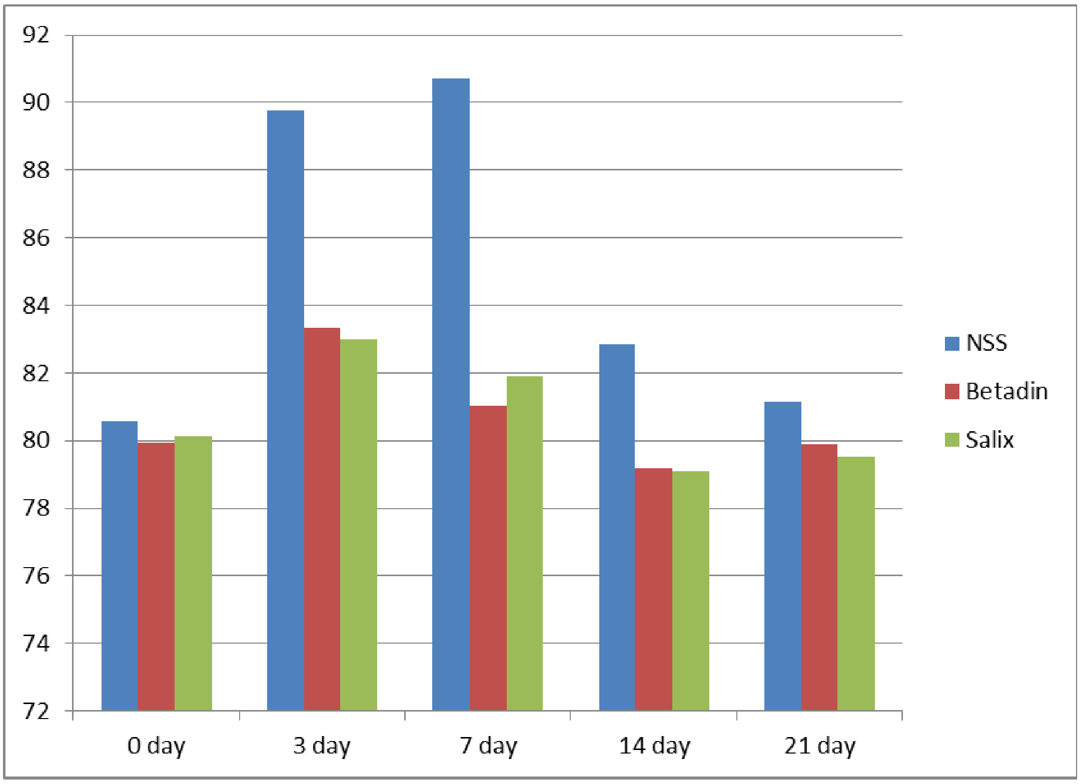


Fig. 23: Effect of different therapy on Creatinine in the rabbits of different groups at different observation intervals

normal physiological limits showing significant (P<0.05) lower value change in II and III groups as compared to group I at different intervals except on day 21.

TABLE 18: THE MEAN±SE VALUES OF CREATININE IN THE RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	80.56 ± 0.72^{aA}	89.76 ± 0.99^{bC}	90.71 ± 1.04^{bC}	82.85 ± 2.37^{bB}	81.15 ± 0.49^{aA}
II	79.93 ± 1.38^{aA}	83.35 ± 3.39^{aB}	81.05 ± 1.83^{aAB}	79.18 ± 2.07^{aA}	79.91 ± 2.07^{aA}
III	80.13 ± 1.36^{aAB}	83.00 ± 2.19^{aC}	81.91 ± 2.28^{aBC}	79.10 ± 1.93^{aA}	79.50 ± 1.08^{aA}

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.2.1.1. Percent Score of wound edges (Appearance of wound) in different rabbits of different groups at different observation intervals

Group No.	Score No.	Observation Intervals (Days)				
		0	3	7	14	21
I	0				33.33	33.33
	1		100	100	66.66	66.66
	2	100				
	3					
	4					
II	0				100	100
	1		50	100		
	2	100	50			
	3					
	4					
III	0			50	100	100
	1		100	50		
	2	100				
	3					
	4					

Wound Edges
0 = Indistinct, diffuse, outline not visible.
1 = Distinct, outline clearly visible, attached with wound base.
2 = Well-defined, not-attached to wound base.
3 = Well-defined, not-attached to wound base/rolled under, thickened.
4 = Well-defined, fibrotic, scarred.
n = 6 animals in each group

Table. 19 : Percent Score of wound edges (Appearance of wound) in different rabbits of different groups at different observation intervals

4.2.1.2. Percent Score of Colour of skin surrounding the wound in different rabbits of different groups at different observation intervals

Group No.	Score No.	Observation Intervals (Days)				
		0	3	7	14	21
I	0					33.33
	1				33.33	66.66
	2			33.33	66.66	
	3		100	66.66		
	4	100				
II	0					100
	1		33.33	33.33	100	
	2		66.66	66.66		
	3					
	4	100				
III	0				66.66	100
	1		33.33	33.33	33.33	
	2		66.66	66.66		
	3					
	4	100				

0 = pink or normal
1 = bright red
2 = white or grey pallor
3 = dark red or purple
4 = black or hyper pigmented

Tab le: 20. Percent Score of Colour of skin surrounding the wound in different rabbits of different groups at different observation intervals

4.2.1.3.: Per cent score of Degree of Inflammation in different rabbits of different groups at different observation intervals

Group No.	Score No.	Observation Intervals (Days)				
		0	3	7	14	21
I	0	100				33.33
	1				100	66.66
	2		100	100		
	3					
II	0	100		33.33	100	100
	1			66.66		
	2		100			
	3					
III	0	100			100	100
	1			66.66		
	2		100	33.33		
	3					

Degree of Inflammation
0 = Clean wound
1 = Redness, no swelling, no discharge
2 = Redness, swelling, no discharge.
3 = Redness, swelling, discharge present.
n = 6 animals in each group

Tab le: 21. Per cent score of Degree of Inflammation in different rabbits of different groups at different observation intervals

4.2.1.4.: Percent Score of Extent of Granulation Tissue in different rabbits of different groups at different observation intervals

GROUP NO.	SCORE NO.	OBSERVATION INTERVALS (DAYS)				
		0	3	7	14	21
	0					16.66.
	1				33.33	83.34
I	2			16.66	66.66	
	3		100	83.34		
	4	100				
	0					100
	1		16.66	33.33	100	
II	2		83.34	66.66		
	3					
	4	100				
	0				83.34	100
	1		16.66	33.33	16.66	
III	2		83.34	66.66		
	3					
	4	100				

Appearance of granulation tissue
0 = Skin intact, partial skin thickness
1 = Bright, beefy red, 75% -100% of wound filled
2 = Bright, beefy red, < 75% &> 25% wound filled.
3 = Pink or dull red less or equal to 25% of wound healed.
4 = No granulation tissue.
n = 6 animals in each group

Tab le: 22. Percent Score of Extent of Granulation Tissue in different rabbits of different groups at different observation intervals

4.2.1.5: Percent Score of Extent of Cicatrization in different rabbits of different groups at different observation intervals

GROUP NO.	SCORE NO.	OBSERVATION INTERVALS (DAYS) 0	3	7	14	21
I	0					16.66
	1					83.34
	2				100	
	3			100		
	4		100			
	5	100				
II	0				100	100
	1			66.66		
	2			33.33		
	3		50			
	4		50			
	5	100				
III	0				100	100
	1			100		
	2					
	3		50			
	4		50			
	5	100				

0 = Normal skin color.
1 = Fade, thin –out or small white line.
2 = Light pink, smaller and softened scar
3 = Red, larger and thicker areas.
4 = Scab formation
5 = no scar formation
n = 6 animals in each group

Table: 23. Percent Score of Extent of Cicatrization in different rabbits of different groups at different observation intervals

4.2.1.6: Per cent score of Presence of Exudate in different rabbits of different groups at different observation intervals

Group No.	Score No.	Observation Intervals (Days)				
		0	3	7	14	21
I	1					
	2	100			100	100
	3			100		
	4		100			
	5					
II	1				100	100
	2	100		100		
	3		100			
	4					
	5					
III	1				100	100
	2	100		100		
	3		100			
	4					
	5					

PRESENCE OF EXUDATES

1 = NONE
2 = SCANT-WOUND BASE MOIST, NO MEASURABLE EXUDATE
3 = SMALL-WOUND TISSUE MOIST, MOISTURE EVENLY DISTRIBUTED.
4 = MODERATE-WOUND BASE SATURATED
5 = LARGE-WOUND TISSUE BATHED IN FLUID

N = 6 ANIMALS IN EACH GROUP

Table 24: Per cent score of Presence of Exudate in different rabbits of different groups at different observation intervals

Gross Observations:

Creation of excisional wounds resulted in variable extent of bleeding and formation of clot. The clot was dried and formed a cover over the wounds, which rendered the evaluation of colour of granulation tissue difficult. The scab, which covered the underlying granulation tissue, was detached in majority of the wounds after 14^{th} post-wounding days. The scar became paler with passage of time, which indicated stage of maturation.

Gradual decrease in the size of the wounds and increase in percent wound contraction (healing) was recorded in all the groups up to day 21 post-wounding. It was interesting to note on day 7 after post-wounding that there was appreciable reduction in size of wound treated with salix and betadin as compared to control group/wounds. Furthermore, complete filling of the wound with granulation tissue without scab and demonstrable wound contraction was noticed in salix on day 14 post-wounding that betadin and normal group. Complete epithelialization and closure of salix and poviodone iodine treated wouds could be distinguished on day 21 and percentage healing 100.00% and 95.66% respectively was observed, whereas in NS complete healing was noticed in only 61.00% of wounds.

4.2.2. Morphometry of wound

4.2.2.1. Wound size

The Mean±SE values of wound size (cm^2) in animals of different groups at different observation intervals are depicted in Table-25 and Fig. 24. Post wounding a significant ($p<0.05$) decreasing trend in the area of wound size was noted in all the groups on all observation intervals. The complete closure of cutaneous excisional wounds was noted by day 21 in the animals of group III.

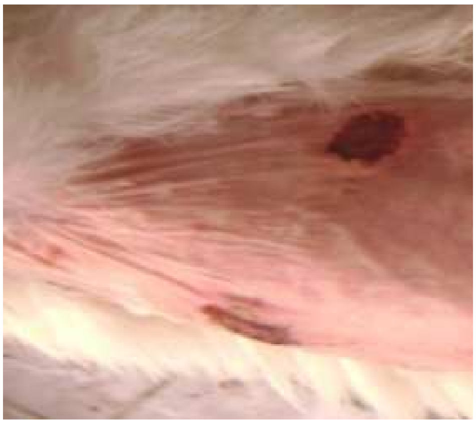
NSS

Betadin

Salix

Plate 1: Gross appearance of wounds of different rabbits of different groups on day 0

NSS



Betadin

Salix

Plate 2: Gross appearance of wounds of different rabbits of different groups on day 3

NSS

Betadin

Salix

Plate 3: Gross appearance of wounds of different rabbits of different groups on day 7

NSS

Betadin

Salix

Plate 3: Gross appearance of wounds of different rabbits of different groups on day 7

NSS

Betadin

Salix

Plate 4: Gross appearance of wounds of different rabbits of different groups on day 14

NSS

Betadin

Salix

Plate 5: Gross appearance of wounds of different rabbits of different groups on day 21

Comparison among the groups revealed significant ($p<0.05$) decrease in the wound size in the animals of treated groups (2 and 3) from those of control group (1) on all corresponding observation intervals. Furthermore the wound size value was significantly ($p<0.05$) lower in the animals of groups II and III from those of group I on all corresponding observation intervals from day 3 to 21 post wounding.

Table 25: The mean±SE values of wound size (cm2) in the rabbits of different groups at different observation intervals

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	2.50±0.04aE	1.76±0.08bD	1.33±0.05cC	1.05±0.08cB	0.62±0.04cA
II	2.47±0.04aE	1.45±0.05aD	0.83±0.01bC	0.69±0.01aB	0.09±0.04bA
III	2.51±0.02aE	1.50±0.06aD	0.73±0.01aC	0.38±0.04aB	0.00±0.00aA

Figures with different superscript (capital letters) differ significantly ($P < 0.05$) between days within the groups
Figures with different superscript (small letters) differ significantly ($P < 0.05$) between groups
n = 6 animals in each group

4.2.2.2. Percentage healing

The Mean±S.E values of percentage healing (%) in animals of different groups at different observation intervals are depicted in Table-26 and Fig-25. Post wounding the percentage healing increased significantly ($p<0.05$) in all groups, at all observation interval with the result cent percent healing was achieved in the animals of group III by the end of the observation period. The healing was still incomplete in the animals of group I and II by the end of the observation period. Comparison among the group showed significant ($p<0.05$) increase in percentage healing of all treated wounds (groups II and III) as compared to control wounds

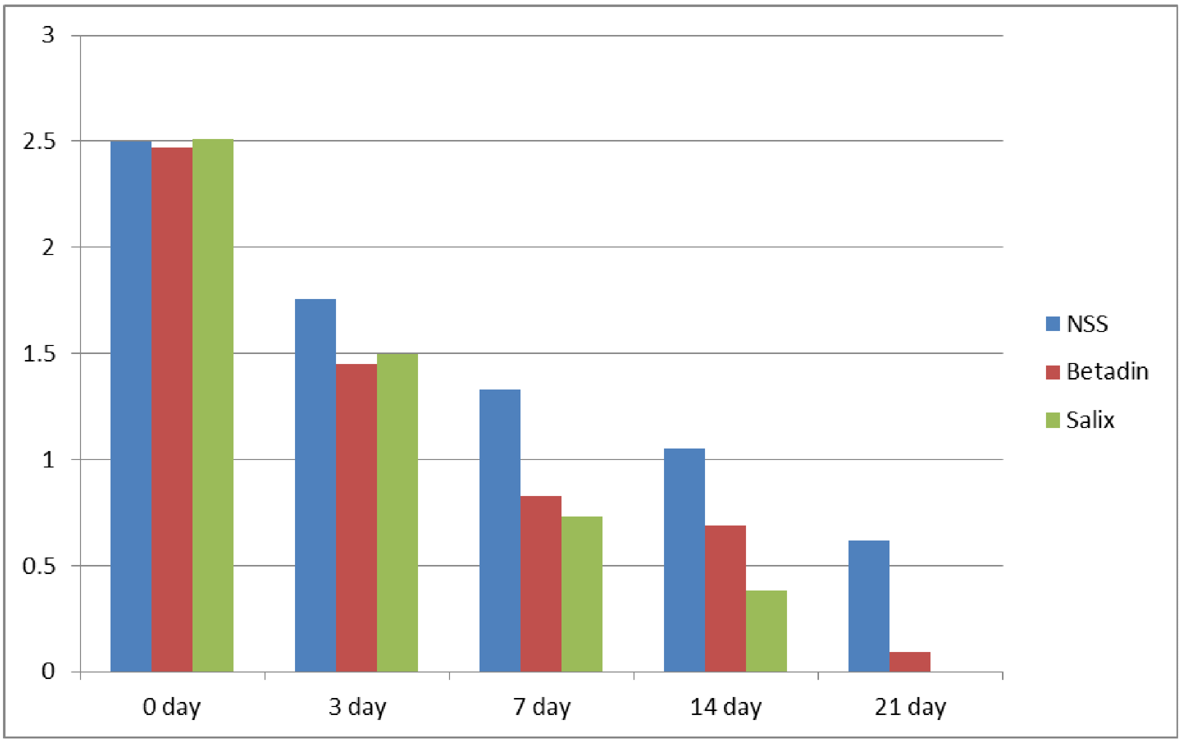


Fig: 24: Effect of different therapy on wound size (cm^2) in the rabbits of different groups at different observation intervals

(group I). Among treated groups percent wound healing was significantly (p<0.05) faster in the animals of group III as compared to the animals of group I and II.

TABLE 26: THE MEAN±SE VALUES OF PERCENTAGE HEALING (%) IN THE RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	$0.00±0.00^{aA}$	$12.50±0.54^{aB}$	$25.50±1.04^{aC}$	$51.83±0.75^{aD}$	$61.00±1.67^{aE}$
II	$0.00±0.00^{aA}$	$15.00±0.89^{bB}$	$45.50±1.04^{bC}$	$71.83±0.75^{bD}$	$95.66±1.63^{bE}$
III	$0.00±0.00^{aA}$	$16.00±2.09^{bB}$	$55.50±1.04^{cC}$	$81.83±0.75^{cD}$	$100.00±0.00^{cE}$

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.3.1 Total Bacterial count (cfu/ml)

The mean±SE values of Total Bacterial count before and after therapy have been shown in table 27 and fig. 26.

A continuous progressive decrease in Total Bacterial count level was noticed in all the groups till day 21. The decrease from the base values was

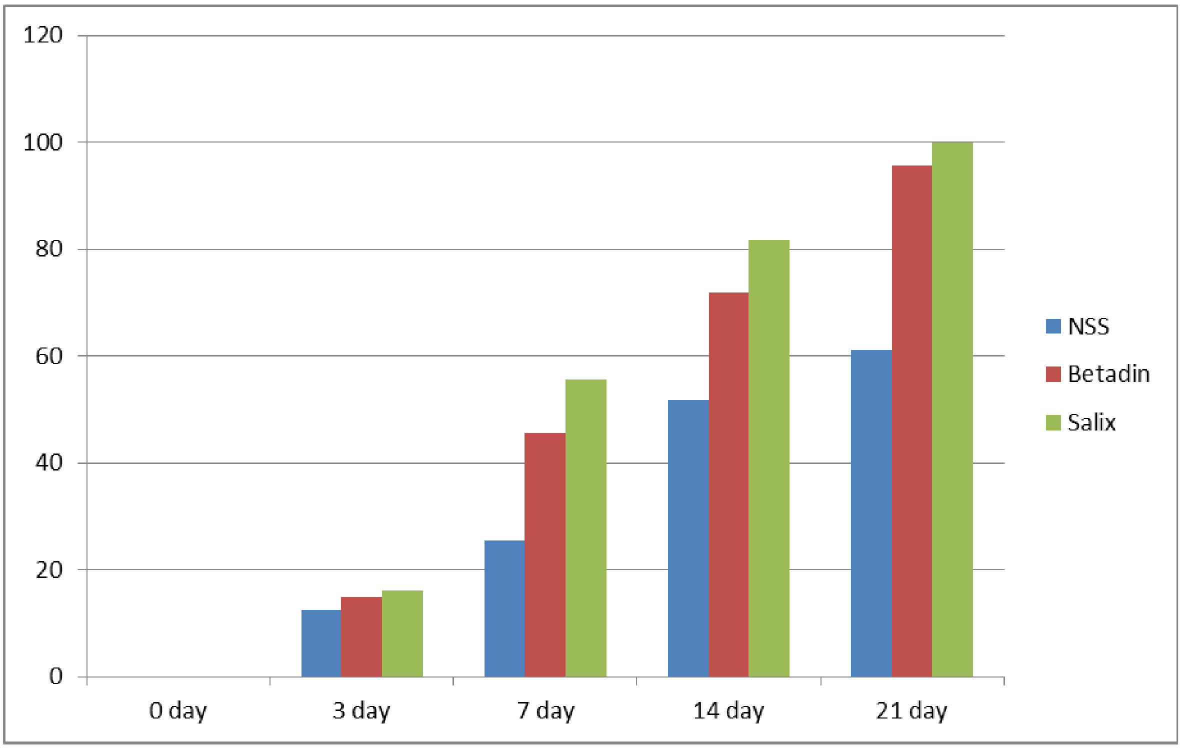


Fig. 25: Effect of different therapy on percentage healing (%) in the rabbits of different groups at different observation intervals

significant (P<0.05) in all groups from day 3 as compared to the base value. In comparison to group I, low levels of Total Bacterial count was recorded in groups II and III and the values were significantly (P<0.05) lower in group III followed by group II at different intervals.

TABLE 27: THE MEAN±SE VALUES OF TOTAL BACTERIAL COUNT IN THE RABBITS OF DIFFERENT GROUPS AT DIFFERENT OBSERVATION INTERVALS

Group	Observation Intervals (Days)				
	0	3	7	14	21
I	1.76 ± 0.13^{aE}	1.25 ± 0.20^{bB}	0.75 ± 0.20^{cC}	0.46 ± 0.08^{bB}	0.07 ± 0.00^{bA}
II	1.55 ± 0.28^{aD}	1.10 ± 0.16^{bC}	0.53 ± 0.22^{abB}	0.20 ± 0.08^{aA}	0.01 ± 0.00^{aA}
III	1.58 ± 0.16^{aD}	0.78 ± 0.21^{aC}	0.40 ± 0.08^{aB}	0.13 ± 0.05^{aA}	0.01 ± 0.00^{aA}

Figures with different superscript (capital letters) differ significantly (P < 0.05) between days within the groups
Figures with different superscript (small letters) differ significantly (P < 0.05) between groups
n = 6 animals in each group

4.3.2. Photographic evaluation

Photographic evaluation of excisional wounds of rabbits was performed on 0, 3, 7th, 14th and 21st day after surgery. The salient observations of the photographic observation suggest that all the excisional wounds aseptically created were almost equidimensional on day '0' (immediately after surgery) (Plate 1-5).

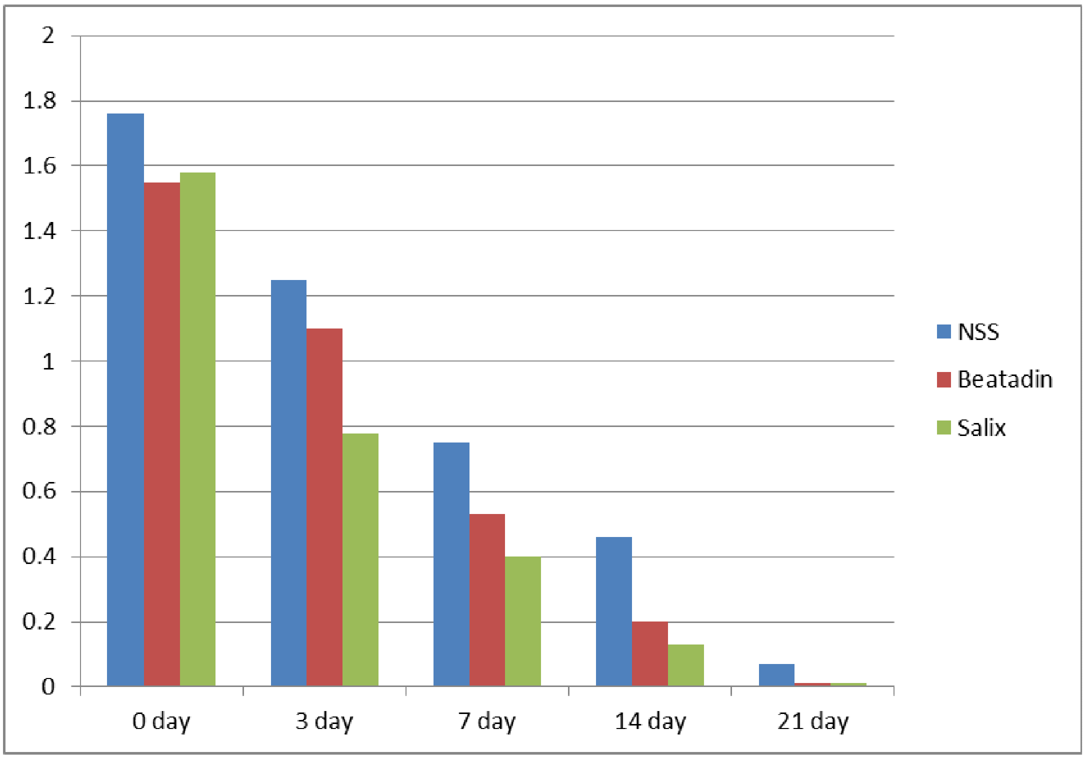


Fig. 26: Effect of different therapy on Total Bacterial count (cfu/ml**) in the rabbits of different groups at different observation intervals**

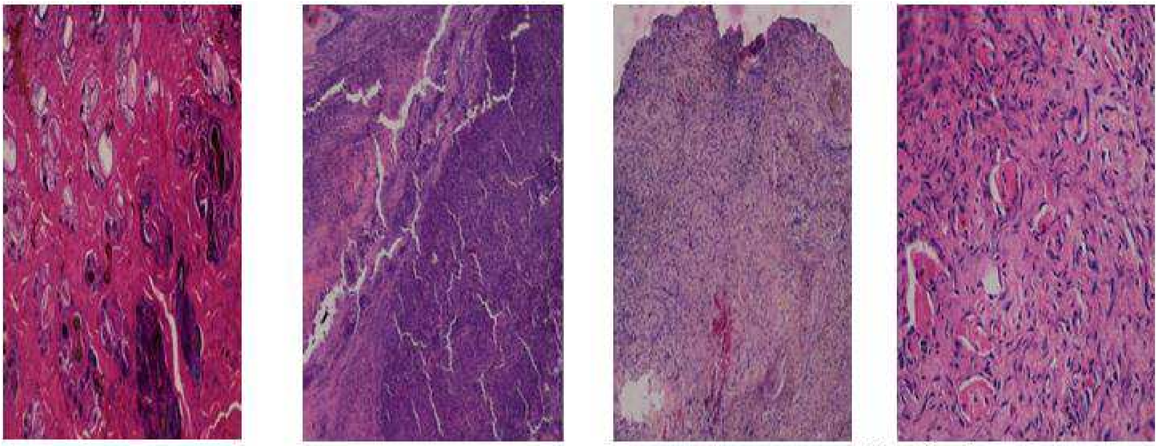
4.3.4 Histo-morphological evaluation

By day 7th post-wounding, granulation tissue associated with admixture of mononuclear and polymorphonuclear cells was seen in salix treated wounds which was thicker than adjacent skin (Plate 10-11). The poviodone iodine treated wounds showed mild to moderate inflammatory reaction with predominant heterophilic infiltration (Plate 8-9), underneath the scab, whereas wounds treated by NSS group showed necrosed tissue having pus cells underneath the scab (Plate 6-7).

By day 14 post-wounding salix treated wounds showed marked granulation tissue associated with vascularisation and heterophilic infiltration (Plate 10-11) The poviodone iodine treated wounds showed granulation tissue with fibroblasts proliferation varying moderate to predominant (Plate 8-9), whereas wounds treated by NS group showed severe heterophilic infiltration (Plate 6-7).

By day 21 post-wounding, wounds treated with salix, showed no inflammatory reaction had denser and thicker collagen fibers in granulation tissue and are partial to complete epithelialization tendency to form hair follicle similar to normal skin (Plate 10-11). The poviodone iodine treated wounds dense collagen deposition with partial epithelialization was observed (Plate 8-9), whereas wounds treated with NS showed predominant amount of collagen deposition associated with partial to complete but immature epithelialization (Plate 6-7).

0 day | 7 day | 14 day | 21 day



Normal skin

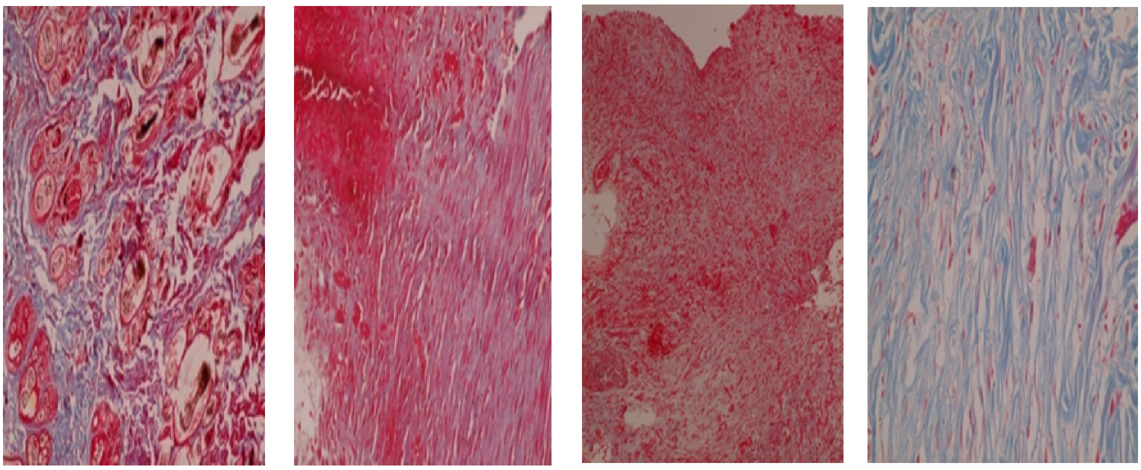
Necrosed tissue having pus cells

Severe heterophilic infiltration

Predominant amount of collagen deposition associated with partial to complete but immature epithelization

Plate 6: Histomorphological observations of wounds of different rabbits of Normal Saline Solutioin (NSS) treated on different intervals (H&E10 x)

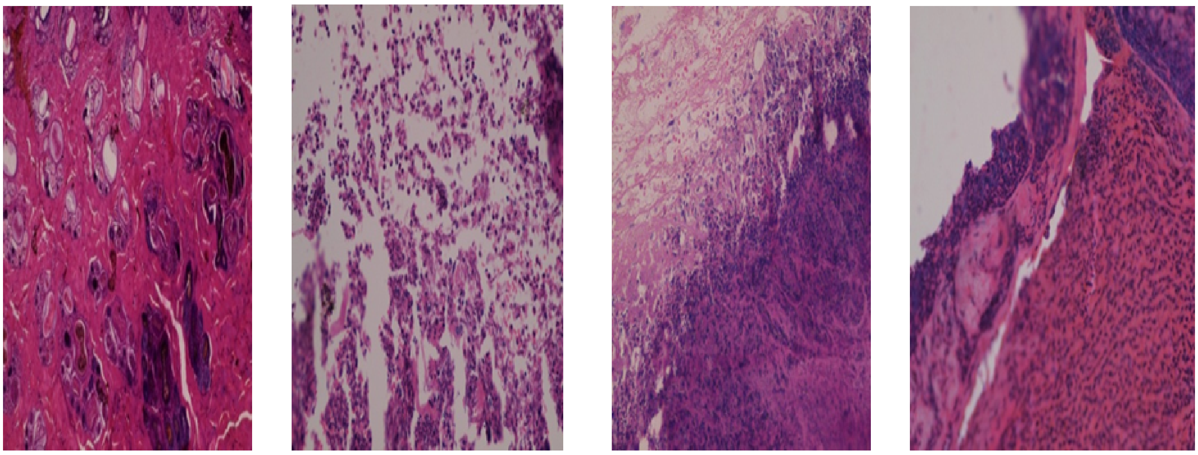
0 day | 7 day | 14 day | 21 day



Normal skin | Necrosed tissue having pus cells | Severe heterophilic infiltration | Predominant amount of collagen deposition associated with partial to complete but immature epithelization

Plate 7: Histomorphological observations of wounds of different rabbits of Normal Saline Solutioin (NSS) treated on different intervals (Masson's Trichome, 10 x)

0 day | 7 day | 14 day | 21 day



Normal skin

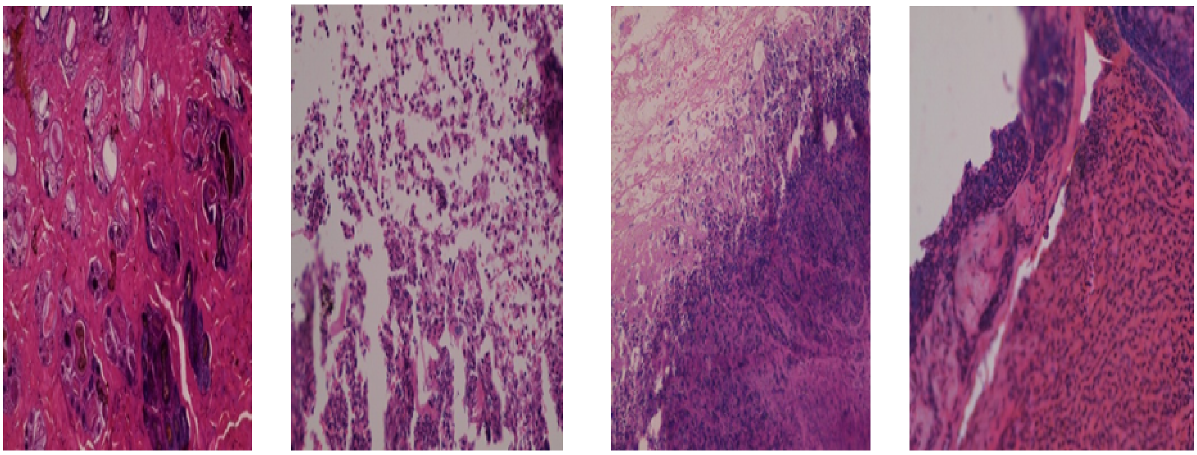
Inflammatory reaction with predominant heterophilic infiltrations

Granulation tissue with fibroblastic proliferation varying moderate to predominant

Dense collagen deposition with partial epithelisation

Plate 8: Histomorphological observations of wounds of different rabbits of Povidone Iodine (Betadin) treated on different intervals (H&E, 10 x)

0 day | 7 day | 14 day | 21 day



Normal skin

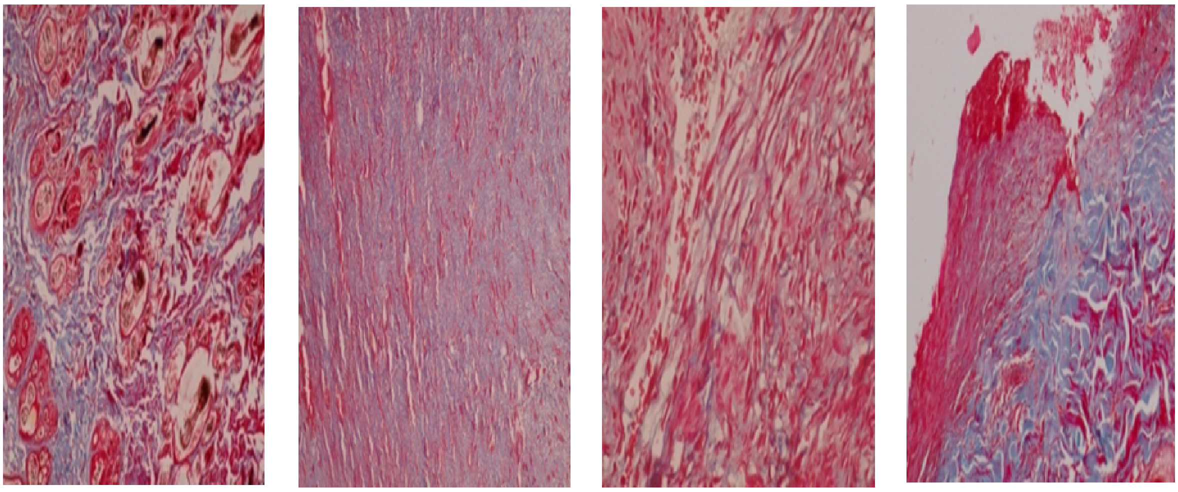
Inflammatory reaction with predominant heterophilic infiltrations

Granulation tissue with fibroblastic proliferation varying moderate to predominant

Dense collagen deposition with partial epithelisation

Plate 8: Histomorphological observations of wounds of different rabbits of Povidone Iodine (Betadin) treated on different intervals (H&E, 10 x)

0 day | 7 day | 14 day | 21 day



Normal skin

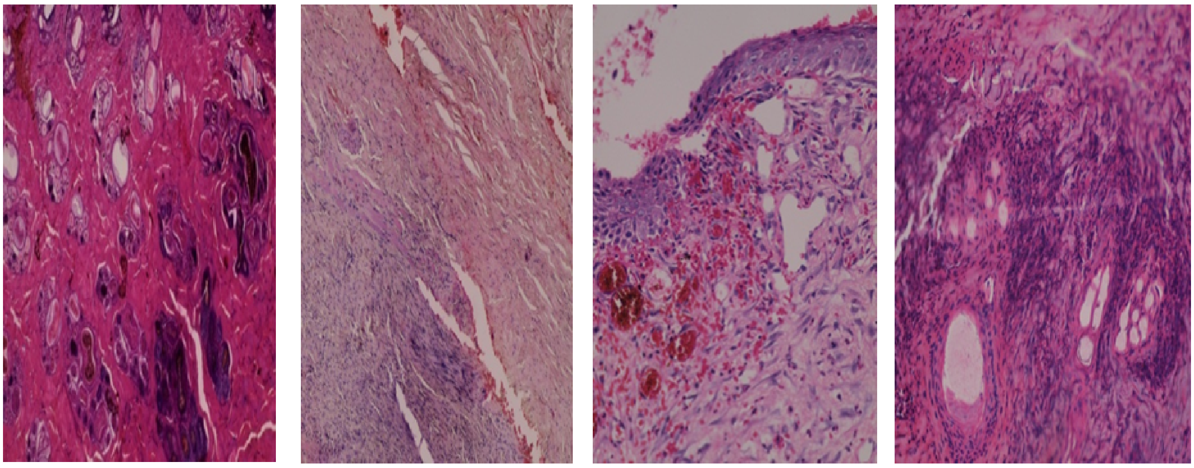
Inflammatory reaction with predominant heterophilic infiltrations

Granulation tissue with fibroblastic proliferation varying moderate to predominant

Dense collagen deposition with partial epithelisation

Plate 9: Histomorphological observations of wounds of different rabbits of Povidone Iodine (Betadin) treated on different intervals (Masson's Trichome, 10 x)

0 day | 7 day | 14 day | 21 day



Normal skin

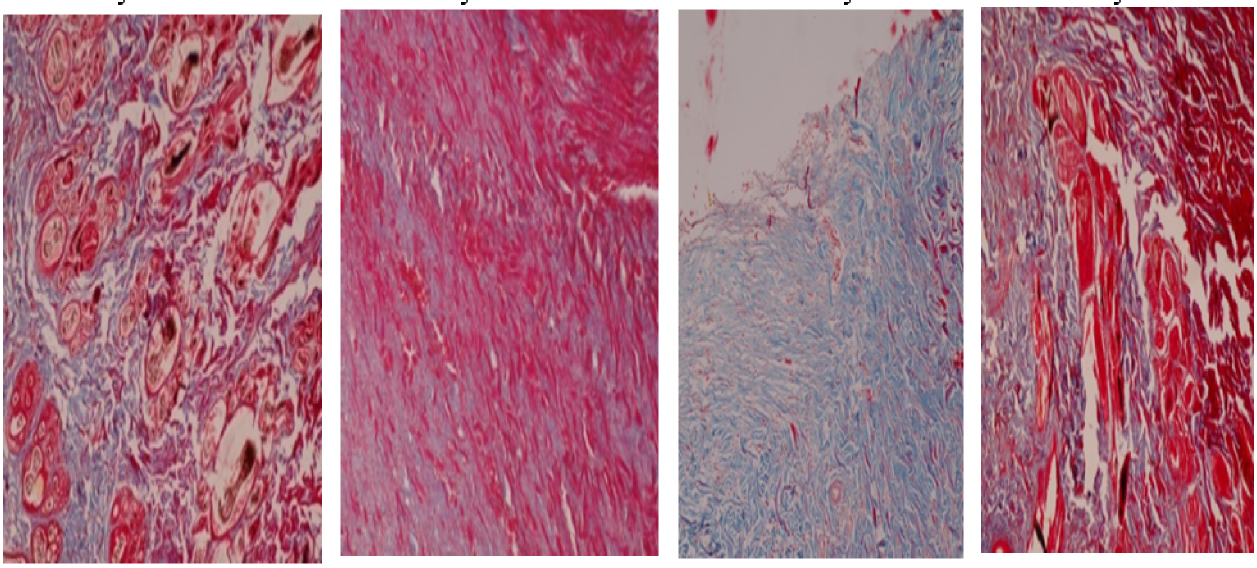
Granulation tissue associated with admixture of mono-nuclear and poly morpho-nuclear cells

Marked granulation tissue associated with vascularisation and heterophilic infiltration

Partial to complete epithelisation tendency to form hair follicle

Plate 10: Histomorphological observations of wounds of different rabbits of Salix treated on different intervals (H&E, 10 x)

0 day | 7 day | 14 day | 21 day



0 day	7 day	14 day	21 day
Normal skin	Granulation tissue associated with admixture of mono-nuclear and poly morpho-nuclear cells	Marked granulation tissue associated with vascularisation and heterophilic infiltration	Partial to complete epithelisation tendency to form hair follicle

Plate 11: Histomorphological observations of wounds of different rabbits of Salix treated on different intervals (Masson's Trichome, 10 x)

4.6. Time required for wound healing

Wounds dressed with *salix acmophylla* leaves ointment (group III) showed considerable signs of full thickness skin wound healing and significantly (*P<0.05*) healed earlier in 17.83 days followed by poviodone iodine treated wounds (group II) in 19.00 days than wounds dressed with sterile normal saline solution (group I) in 20.83 days (Table 28).

Table 28: Mean ± S.E. of time required for wound healing

Group	Healing time (days)
I	20.83 ± 0.40^{a}
II	19.00 ± 0.89^{b}
III	17.83 ± 0.75^{c}

Means with different superscripts within a column were statistically significantly different (*P<0.05*)

Chapter – 5

DISCUSSION

In present study, four full thickness excisional skin wounds (1.5 cm × 1.5 cm), 2.5 cm apart from each other were created (dorsal spine of thoraco-lumbar region) on each of 18 rabbits of either sex and weighing 1.8 to 2.5 kg under standard anesthetic protocol to evaluate the wound healing potential of *salix acmophylla* leaves ointment with povidone iodine (negative control) and sterile normal saline solution (positive control). The total number of wounds evaluated in the study was 72 and thus each treatment groups was evaluated on 24 wounds.

Wound is defined as the disruption of cellular and anatomic continuity of tissue (Bennet, 1988). The process of wound healing consists of integrated cellular and biochemical events leading to re-establishment of structural and functional integrity with regain of strength of injured tissues. The events include coagulation, inflammation, formation of granulation tissue and tissue re-modelling (Lynch *et al.*, 1987; Savant and Shah, 1998). Wound healing, has a continuing challenge in rehabilitation/regenerative medicine. Rapid and uncomplicated wound healing, always a primary goal of surgery, is practically desirable for large wounds or wound over vital structures because failure of wound repair is potentially catastrophic (Bohling *et al.*, 2006). Despite some recent advances in understanding its basic principle, problem in wound healing continues to cause significant morbidity and mortality.

In India, medicines based on herbal origin have been the basis of treatment and care for various diseases (Biswas *et al.*, 2003). Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation and infection etc. (Mukherjee, 2000). More than 80% of the world's population will depends upon traditional medicine for various skin diseases (Babu *et al.*, 2002). Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process (Purna and Babu, 2000).

Majority of the people in Kashmir valley on medicinal plants to find treatment for their minor, even in some cases major diseases. In most instances, certain plant species are considered specific for a particular illness but occasionally they have mixed usage. Though medicinal plants from wild are important source of income for local communities, but if not properly managed, this may lead to the destruction of habitat and in return extinction of species (Shinwari, 2010). Starting the pre- historic era to date, people healed themselves with local plants remedies. In the recent days, one can observe an international drift of significance in the long established structure of medicines. Evaluation of therapeutic herbs has turned into a latent basis of biodynamic substances of curative value (Mahmood et al., 2011). Rapid urbanization and unplanned exploitation have resulted in loss of medicinal plant species. It is therefore, imperative to find ways to encourage practices for promoting conservation. There is need of coordination and cooperation among various agencies such as forest department and the pharmaceutical firms interested in the utilization of these medicinal plants and to initiate regeneration work in degraded or areas devoid of vegetation. By doing so, we can change the economic and social conditions of the local inhabitants positively (Qureshi et al., 2011).

A surgeon routinely encounters different types of wounds with or without loss of tissue. The main aim of early healing of wounds. Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral and parenteral) in an attempt to aid wound repair (Savant and Shah, 1998; Rains and Mann, 1988). Antibiotics are commonly used topically for preventing bacterial colonization. These antibacterial agents have certain limitations that may cause tissue destruction by irritation or allergy (Nagesh et al., 1999). It is, therefore, important that the dressing used should not promote the growth of harmful bacteria and at the same time it should not have deleterious effect on the tissue. Consequently there exists a need for new agents which may be useful in proper wound management. In this direction a number of herbal products are being investigated. Salix babylonica leaves extracts used in drinking water on performance and heat tolerance of broiler chickens during heat stress (Al-Fataftah and Abdelqader, 2013). In the current study salix acomophylla leaves ointments were used for treating the wounds (1.5 cm x 1.5 cm) in the thoroco-lumbar region in adult's rabbits. Sterile normal saline solution and povidone iodine were used in control groups. The effects of these treatments were evaluated on the basis of clinical, hemato-biochemical, histo-pathological and bacteriological observations. The results obtained are discussed accordingly under the following headings:

5.1. Animal detail

Many animals have been used during previous studies to evaluate wound healing such as buffalo calves (Kumar and Tyagi, 1972), cow calves (Aakhoon, 2001), dogs (Hamamoto *et al.*, 2009), mice (Robertson *et al.*, 1974; Eurides *et al.*, 1998), rats (Chitra *et al.*, 1998), guinea pigs (Shukla *et al.*, 1999), rabbits (Madhu, 2012; Handoo *et al.*, 2014,Sandeep et al., 2015). Rabbits provide useful models for studies designed to develop new bandage materials (Cangul *et al.*, 2006) or to investigate novel therapies, such as cell therapy (Lee and Moon, 2003; Sandulache *et al.*, 2003; Sumiyoshi *et al.*, 2004), Platelet concentrates (Lee *et al.*, 2008) and laser therapy (Brosh *et al.*, 2004; Simhon *et al.*, 2004). These comparative analyses are based on observation of re-epithelialization and wound contraction (Bujan *et al.*, 2006). In the present study rabbits were used for evaluation of wound healing efficacy of *Salix acmophylla*. The rabbits used were of either sex, aged between 1.8-2.5 kg. Animals were found easy to handle and wounds were created without any problem. These findings match with those of Ramos *et al.* (2008) findings. Larger wounds of 1.5 cm x 1.5 cm dimensions could be created due to availability of larger body surface area of animals owing to their more body size.

5.2. Wound model

Different wound models have been used for evaluation of wound efficacy in the previous studies which include incisional (Kakali- Saha *et al.*, 1997; Asif *et al.*, 2007), excisional (Kakali- Saha *et al.*, 1997; Asif *et al.*, 2007; Gupta *et al.*, 2010, Madhu, 2012; Handoo *et al.*, 2015), dead space wound (Asif *et al.*, 2007; Nayak *et al.*, 2009), suppurative wounds (Pradhan, 1995), full-thickness wounds (Chitra *et al.*, 1998, Lemo *et al.*, 2010; Nisbet *et al.*, 2010), partial thickness wounds (Lynch *et al.,* 1987; Lynch *et al.,* 1989; Haupt and Chvapil, 1990; Stephen *et al.,* 2001). Full-thickness wounds proved to be better and easier to create. Harvested wounds can be examined histologically for both the epithelial gap and granulation bed characteristics (Wong *et al.,* 2011). Different researchers have used different techniques and instruments for creation of wounds e.g., metal marker (Aakoon, 2001, Handoo *et al.,* 2015), laser technique (Manolis *et al.,* 2007), tracing paper (Madhu, 2012). Also different shapes of wounds have been created in previous studies which include triangular, circular, rectangular, square shapes by different workers. Circular wounds, however, contracted at a perceptibly slower rate; because it is argued that they present a greatest resistance to the contractile forces. In order to have a suitable model for evaluation of Salix acmophylla on wound healing in present study full-thickness 1.5 x 1.5 cm square excisional of wounds e.g., epidermis and dermis (Lemo *et al.,* 2010). 2.5 cm apart and two wounds on each on each side e.g. total four wounds per animal. Similar methods were used by Akhoon (2001) in buffalo calves creating excisional wounds could be estimated in nature. These observations match with those of Billinghama and Russell (1956) who also observed that the contracture of cutaneous wounds in rabbits proceeded very nearly to completion. The walking ability was not hindered by wound induction on back portion. All the animals were comfortable during the test period. The animals were kept in sterna recumbency easily reproducible position, the outline of the intended wound was first marked accurately on the prepared skin using an instrument made according to the required measurements by placing it on position and marking the outline using BP blade. This was then incised down through the epidermis and dermis. The full thickness of the skin within the incision was then carefully stripped away by sharp dissection from its underlying muscle and discarded (Billinghama and Russell (1956). In the rabbit, the healing process involves maximum wound contraction, prior to the initiation of the cell migration and matrix remodelling (Abramov *et al.,* 2007; Abramov *et al.,* 2006).

5.3. Anaesthetic protocol

Few safe and effective anesthesia regimens have been described for use in rabbits, partially because of the susceptibility of this species to sometimes fatal respiratory depression. Although inhalant anesthetics are generally safer than injectable anesthetics, their use may be limited by lack of equipment or facilities. Different anesthetic regimes have been evaluated in rabbits under laboratory conditions. Many drugs are used intramuscularly in rabbits in combinations such as Ketamine-Xylazine, Xylazine-ethyl-(1-methyl-propyl) malonyl-thio-urea salt, ketamine-EMTU, Xylazine-acepromazine-ketamine and ketamine-chloral hydrate are used in combination with one another (Hobbs *et al.*, 1991).

Xylazine have proven to be safe anaesthetic agent when used in combination with ketamine to induce short periods of anaesthesia (Thurmon *et at.*, 1996). Ketamine produce good analgesia and less locomotor activity seen during recovery (White *et al.*, 1980). Although ketamine initially produces depression of heart rate and respiratory rate, but values return back to normal after 15-20 minutes. Combination of xylazine and ketamine improves and prolongs muscle relaxation and analgesia. The emergence is also smooth (Lin, 1996)..

In present study Ketamine- Xylazine combination was used. Ketamine at the dose rate of 50mg/kg body weight intramuscularly and Xylazine at the dose rate of 10 mg/kg body weight intramuscularly. This combination used gave the wanted results. The dose used created the suitable depth and duration of anesthesia as required for the surgical manipulation. The animal remained anesthetised up to the completion of the surgical procedure (30 minutes) and no maintenance dose was required. The combination produces adequate analgesia and muscle relaxation. There was no complication of anesthesia and surgery observed, as the standard protocol was followed in rabbit excisional skin wounds, which is in accordance with previous studies conducted by Handoo *et al.* (2014).

5.4. Evaluation of medicaments on animals

5.4.1. Physiological analysis

5.4.1.1. Rectal temperature:

In the present study physiological parameter *viz*: rectal temperature was within normal range 38.60-39.76 ^{0}C as also reported by Kumar and Chauhan (2003); Meier (2007). In rabbits temperature is recorded per rectal. Rabbits are sensitive to environmental temperature changes. Environmental temperature of 15-21^{0}C will allow the conscious animal to maintain normal body temperature of 38.5-39.44 ^{0}C (Batchelor, 1999; Brewer and Cruise, 1994). They should be protected from environmental temperature of below 4 ^{0}C, and show signs of heat stress above 28^{0}C. In the present study values were normal indicating the animals were healthy. However, the base values were at lower end of range which could be due to decreased environmental temperature as the present study was carried out during winter months (October-April). All the groups showed increasing trend in the value of temperature from 48-72 hours after wounding. Increase in temperature is a feature in every instance of inflammation following tissue damage. The inflammatory response usually reaches its maximum at 48-72 hours post-operatively (Vegad, 2004). The occurrence of temperature within normal range during study period can be attributed to the use of *salix* as it has an inhibitory effect on prostaglandins. In rabbit's renal medulla, *salix* inhibited the metabolism of arachdonic acid to prostaglandins E2 and F2 and thromboxane in a dose dependent fashion (Guo *et al.*, 1989). Also in wounds treated with povidone iodine the animals did not show significant increase after the inflammatory period, this could be because of the absence of infection at the wound site, attributed to the fact that povidone iodine has anti-bacterial activity (Berkelman *et al.*, 1982).

5.4.1.2. Heart rate

In the present study physiological parameter *viz*: heart rate was normal range from 186-265 beats/minute reported by Kumar and Chauhan (2003), (Meier, 2007). Heart rate is a complex physiological variable that can be varied fear, stress, degree of anaesthesia and vascular volume (Lin *et al.*, 1993). In current study the base values were normal indicating the animals were healthy. Then the values in all the groups followed different trend. All the groups showed an increased heart rate value than the base values. Rabbits being timid animals, even subtle handling of animal for recording the clinical parameters can increase the heart rate. The heart rate of rabbit is rapid and at times may exceed normal range (Praag, 2003). In wounds treated with *salix* leaves ointment showed an increase heart rate may be attributed Salix, which showed an excitatory effect.

5.4.1.3. Respiration rate

The normal range of respiration rate of rabbits is between 30-60 breaths per minute depending upon the size and age of the rabbits (Lesa, 2008). The respiratory rate is influenced by environmental conditions, temperature, pain, fever and stress (Sjaatad *et al.,* 2005). In the current study normal base value indicated the animals were healthy. The values then showed an increase on day 1 post-wounding. This could be attributed to the increased temperature, pain and stress in the animals. Also it has been stated that during the period of stress, rabbits increase their respiratory rate from normal range to 200 -250 beat per minute (Thurmon *et al.,* 1996). Similar respiratory rates were observed in rabbits of the all the groups indicating the action of analgesic in controlling pain as well as uniformity in the husbandry practices.

5.4.2. Haematological analysis

The physiological and pathological conditions of animals can be assessed by the evaluation of hematological and biochemical analyses of the blood (Bush 1991). Haematogical parameters are considered as markers for presence of inflammation, infection and stress especially TLC and DLC.

5.4.2. 1. Haemoglobin (Hb)

Haemoglobin content is an important parameter among the haematological analysis. It gives a good indication of oxygen transportation capacity of the animal and hence the health status of the animal (Babek *et al.,* 2012). In present study the base values were within the normal range which is indicative of good health due to proper management of animals. The groups treated with saline solution and betadin ointment showed significanlty lower values as compared to the corresponding base values on day 3 and 7 and then there were significantly higher values on day 14 and 21. The values in wounds treated with *salix* group remained well within normal range, at all the corresponding observation intervals which could be attributed to the use of *salix,* as Willow bark (*Salix alba*) also indicated that catechin was the major constituent responsible for the radical scavenging activity in willow bark preparations to cause a decrease in serum content of lipid peroxidise and increase in the content of super dismutase activity in erythrocytes which leads to the improvement in the haemoglobin content of the animals (Agnolet *et al.*, 2012).

5.4.2. 2. Packed cell volume (PCV)

The hematocrit (PCV) percentage is an important parameter, which besides giving an indication of oxygen transportation capacity also gives an indication of hydration level of the animal (Babek *et al.,* 2012). Normal base value of PCV, recorded in the animals of this study points towards within normal range in all the groups. An increase in the values of PCV up to 7 post-wounding in the animals of all the groups was recorded in the current study and could be attributed to dehydration caused by insufficient intake of water due to pain in animals. From day 3 post-wounding onwards the values fluctuated around the base values in all treated groups. This could be attributing to the proper intake of water owing to decline of stress and pain and manifested by faster healing. This finding corroborated that of Qasim (2011) who recorded an increase in the PCV values of rabbits during increased temperature conditions.

5.4.2. 3. Total Erythrocytic count (TEC)

The interpretation of erythrocytic count give clue about is presence or absence of anemia. The TEC was normal in all the animals before wounding signifying good health status of all the animals. On day 3 post wounding there was decrease in TEC values in all groups which could be attributed to little loss blood during wounding. In groups wounds treated with sterile normal saline solution and povidone iodine ointment however this decrease was significant and continued till day 7 and thereafter started returning to base values. This return was earlier in treated groups on day 14 post wounding. There have been conflicting reports on the number of erythrocytes by the use of herbal drugs. Herbal drugs may contain certain chemical which may lead to erythrocytic destruction (Benjamin, 1978). In present study, *salix* showed significant effect on TEC values in rabbits which started returning to base values earlier. This is on contrary with the findings of Mudhir (2008) who reported no significant effect of Salix on TEC values. Also it has been reported that heat stress decreases the level of ACTH, which, in turn, decreases the values of RBC count (Seley, 1960).

5.4.2. 4. Total Lecucocytic count (TLC)

The leucocytic count may vary in rabbits with diurnal fluctuation, nutritional variations and difference in age, gender and breed (Bollinger *et al.*, 2010). In present stud there was increase in TLC count which was within normal range in all the groups, this increase was up to 7 day post wounding and then started decreasing, however remained well within normal range. The increase in the TLC values may also be attributed to the presence of inflammation and stress. These changes in leucograms could be related to tissue trauma as a result of surgery (Kirov *et al.*, 1979), heat and humidity (Kennedy and Mackay, 1986) and pain and anxiety (Milhorat *et al.* 1987). Leukocytosis tends to result from bacterial infections, trauma, stress and intense exercise. However, in conditions of complete physical and mental relaxation, a basal level is maintained. Present result is in accordance with findings of Mudhir (2008), who found significant increase in TLC values of animals after treating the animals with salix.

5.4.2.5. Differential leucocytic count (DLC)

Differential leucocytic count in a healthy rabbit fluctuated considerably when evaluated over a single month (Mitruka and Rawnsky, 1981).

5.4.2. 5.1. Neutrophils

Neutrophils are the first line of defense (Vegad, 2004). In present study, the neutrophils showed an increase post-wounding reaching maximum on 24-48 hours. An inflammation subsides the neutrophil level started decreasing and returned to normal by the end of study period. The accumulation of neutrophils and its products play an important role in initiating vascular permeability (Campbell, 2004). As a result of which the neutrophils increase and reach a peak around 24-48th day of the inflammatory reaction. In case of wounds treated with *salix* there was a decrease in the values of neutrophils earlier in on day 3 and 21 as compared to control group. This may be attributed to the anti-inflammatory potential of *salix* (Barnes *et al.,* 2002). In clean surgical wounds, neutrophils are undesirable because they delay wound closure and create additional tissue damage (Dovi *et al.,* 2003).

5.4.2. 5.2. Lymphocytes

Lymphocytes are important indicators of infection. They play an important role in humoral antibody formation and cell-mediated immunity. In current study there was a decrease in values of lymphocytes post-wounding which started returning to normal values towards the end of the study period. A low normal lymphocyte concentration is associated with increased rates of infection after surgery and trauma. These facts explain the basis for the maximum values for neutrophils and minimum values for lymphocytes noted in current study from day 3 till day 14 in all groups.

5.4.2. 5.3. Monocytes, Basophils and Eosinophils

Monocytes, Basophils and Eosinophils constitute a very small portion in the total count of leucocytes. Any significant change in such small values may not be considered. However, in present study the monocytes showed an increase on day 1 post-wounding in all groups, but showed a decrease below the corresponding base values on day 2 and 3 viz. in the peak hours of inflammation. This could be transitory decrease in monocytic ocunt. This observation is in consonance with that of Weiss and D'souza (2010) who reported that acute inflammation leads to transient decrease in the monocytic count. The basophils increase at the time of allergic reaction (Vegad, 2004). In current study neither basophils didn't show any significant change. Salix is reported to have an anti-allergic effect (Mastuda *et al.,* 2004), hence no increase in basophils was reported.

5.4.3. Biochemical analysis

5.4.3.1. Total protein and albumin

Proteins are very important molecules in our cells. They are involved in virtually all cell functions. Each protein within the body has a specific role. Some proteins are involved in structural support (Eckersall, 2008), while others are involved in bodily movement, or in defense against germs. Albumin is the most prominent serum protein. It is an acute phase protein. Albumin is the major labile storage reservoir of proteins and as well as being a transporter of its constituent amino acids (Kaneko, 1981). Albumin being a negative acute phase protein, its concentration falls gradually during infections and inflammatory conditions (Anraku *et al.,* 2001). The advent of the inflammatory process including infection, trauma, surgery, burns and other wounds elicits the acute phase response. During this acute phase response, these proteins decline and as such are called a negative acute phase reactants (Posthauer, 2011). The metabolic response to trauma and sepsis involved an increased loss of body proteins (Biolo, 1997). The inflammatory process associated with surgical intervention could be held responsible for continuous post operative decrease in protein and albumin concentrations, because fluids and proteins move into tissue fluids, inducing edema and contributing to a decrease in total protein and albumin (Eckersall, 2008). Also food intake is an important criteria for better healing of wounds. It was reported that the patient's recent food intake is more important in determining the wound healing rate then the patients overall nutritional status (Windsor *et al.,* 1988). During wound healing, the cells, particularly fibroblasts, synthesize and secrete proteins to form scar tissue, while macrophages secrete the growth factors which direct the activity of surrounding cells and, thus, the sequence of wound healing. All of these activities place extra nutritional demands on the patient, and wounds, fistulae and burns also cause an increased loss of protein from the body through exudates. The main protein synthesized in the healing wound is collagen. Proteins in the diet and structural body proteins, if necessary, are broken down into the amino acids, which are then used for cell division and to manufacture the proteins required for scar tissue formation (Casey, 1998). Plasma total protein concentration is significantly affected by the season (Ayyat *et al.*, 1996). In rats, salicylate diffuses rapidly into and out of implanted sponges more rapidly than albumin or naproxen (Doherty *et al.*, 1977), a non- steroidal anti-inflammatory drug that is more strongly bound to plasma proteins than salicylate. The different behaviour of salicylate and naproxen indicates that, in this system, salicylate diffuses into the sponges primarily in the unbound form (Graham, 1988). By contrast, Higgs *et al.* (1987) found that salicylate and aspirin diffuse slowly between plasma and exudates in polyester sponges soaked in carrageenan. Varying rates of diffusion into and out of inflammatory sites should be considered in experiments on the anti-inflammatory effects of the salicylates and related drugs.

In current study the base values of proteins in animals of different gruops, were within normal range and difference among intervals noted was not significant. This could be attributed to the fact that proteins of an individual or of a species are synthesized under genetic control, hence the variations in proteins between individuals, are as on the expected line. Decrease in the values of total protein and albumin from day 1 till day 14 post-wounding was observed in current study. This could be attributed to the inflammatory processes and to decresed food intake due to pain. This decrease in total protein and albumin after wounding could also be due to surgical stress and high temperature at the study region. These findings are in consonance with those of Eskersall (2008) who observed that high temperature and stress especially bone fractures, extensive surgery and crushing injuries is associated with nitrogen loss, leading to decrease in total protein and albumin. Furthermore, there was increase in total protein and albumin towards the end of the study period, this could be attributed to increased synthesis of amino acids and proteins in the remodelling phase of wound healing. Henceforth, better healing is found in *salix* treated wounds were the total protein and albumin levels returned to normal values earlier, infection was least and inflammation subsided earlier.

5.4.3.2. Glucose

In current study on day 3rd and 7th post-wounding the glucose level of wounds treated with *salix* group decreased, which could be attributed to decreased feed intake due to pain in animals of all groups. After day 3rd post-wounding the control group showed minor fluctuations in the values of glucose from the corresponding base value, but remained higher than the base values. This could be due to the transitory hyper-glycaemic effect attributed to cortisol release (Rosin 1981). Cortisol which is release in response to injury, accelerate gluconeogenesis and ketogenesis leading to increased blood glucose levels (Rosin, 1981). In *salix* treated wounds showed decreased values of plasma glucose, this may be attributed to the presence of glycoside E, which have a hypo-glycaemic effect (Li *et al.*, 1997).

5.4.3.3. Blood urea nitrogen (BUN) and Creatinine

Serum blood urea nitrogen and creatinine have been used as important indices for the evaluation of the effects of chemicals on the kidney (Davis and Berndt, 1994). As urea and creatinine represent the two main nitrogenous components that are eventually excreted by the kidney, therefore changes in their levels in the blood stream would reflect the changes in kidney functions (Miller, 1966; Soliman *et al.,* 2000). The presence of increasing urea and creatinine concentration in the blood suggest the inability of the kidney to excrete these products, which further suggest a decrease in glomerular filtration rate (Ovuru *et al.,* 2004). Increase in creatinine levels is more faster that urea levels at the start of disease, and also decrease more quickly when an improvement takes place, thus being a more specific indicator of kidney injury than BUN (Kerr, 2002). Creatinine, the anhydride of creatine, is formed due to fragility in muscle by irreversible non-enzymatic anhydration of creatine phosphate, which is now known to be concerned with the energy mechanism of these tissues and serves primarily as a temporary store of energy. The increase in creatinine may indicate changes in kidney function. Blood urea nitrogen and creatinine evaluation is important to analyze the effect of *salix acmophylla* on kidney function.

Salix has been studied for the management of renal function disorders (Rainsford, 2004) and has been reported to be a nephroprotective drug. Nephorprotective effect of *salix* has been attributed to tannins present in salix which significantly improve BUN abd creatinine, glomerular filtration rate, renal plasma flow and renal blood flow (Thieme, 1968). Salicylate is metabolised to reactive metabolites in rat kidney mitochondria and, to a lesser extent, in liver mitochondria (Kyle and Kocsis, 1986a). With increasing age, there is increased covalent binding of salicylate to the mitochondria of renal cortex. Pretreatment of rats with piperonyl butoxide, an inhibitor of the cytochrome P450 system, decreases both the renal toxicity and covalent binding of salicylate equivalents to renal mitochondria in rats (Kyle and Kocsis, 1986b).

In the current study the concentration of BUN and creatinine was found within normal range. However the BUN values of wounds treated with salix group showed a decreasing trend concentration by the end of the study period as compared to base value. In wounds treated with povidone iodine group BUN value showed an increasing trend on day 3 and 7 post-wounding which decreased to near base values concentration by the end of the study period as compared to base value. In wounds treated with sterile normal saline solution BUN value showed a decreasing trend to near base values concentration by the end of the study period. The creatinine values in all groups showed a significant increasing trend upto day 14 in wounds treated with sterile normal saline solution and up to day 7 wounds treated with *salix* and poviodone iodine, but wounds in all groups values remained non-significant on day 21. This is in accordance with findings of Ovuru *et al.* (2004), who found increase in values of creatinine during stress. Increase in control group may be due to absence of any nephroprotectant and also due to stress in animals post-wounding which caused the increase in values.

5.5. Evaluation of wound

5.5. 1. Clinical evaluation of wound

5.5.1.1. Appearance of wound

The wound edges and the skin surrounding the wound area give important clue about the gross appearance of the wound. In the current study, on day 0 the wound edges were well-defined and not-attached to wound base in any group. Skin surrounding the wound area showed normal colouration in the entire groups throughout the study period. Post-wounding the edges changed from well defined to diffuse and barely visible. These changes were faster in treated groups than in control group. In case of positive control wound treated group povidone iodine solution was used. povidone-iodine also allowed the normal healing of wound edges when applied to the surgical wound and is reported to be safe from the standpoint of wound healing, and also decreases the number of wound infection (Viljanto, 1980). *Salix* treated wound showed faster improvement in wound edge status as compared to control group. There is a priority of nature inherent in healing. Factors critical to survival such as phagocytosis, blood flow and surface covering occur early in wound healing. The wounds in which the edges are not together because of extensive tissue loss or large surface areas those wounds heal by secondary intention. These wounds heal slowly through the process of granulation and epithelialisation (Shipperley and Martin, 2002).

5.5.1.2. Degree of inflammation

Inflammation is an immune response to cellular/tissue injury or infection by pathogens. It is clinically characterized by features such as redness, warmth, swelling, and pain. The process itself is not considered a disease, but failure to contain it and successfully resolute it in a timely fashion results in exacerbation of tissue damage and modulation of cell signaling pathways (Serhan and Savill, 2005). Inflammation involves a portfolio of cellular and molecular components collectively referred to as inflammation mediators.

If there is no inflammation, healing does not begin. If too little inflammation occurs, healing is slow. If too much inflammation occurs, excessive scar is produced (Hardy, 1989). In current study all the groups showed signs of inflammation on day 3 post-wounding; eventually it subsided in all the groups by the end of the study period. This was faster in *salix* treated wounds than the povidone iodine treated wounds followed by control group. This could be attributed to anti-inflammatory effect of *salix* (Barnes *et al.*, 2002).

5.5.1.3. Extent of granulation tissue

It is the primary means of repair for all vertebrates. Special cells in our body respond to injury by forming a collagenous “glue”. This body glue is called granulation tissue (Hardy, 1989). In full-thickness wounds the epidermis can resurface from the margins only after adequate granulation tissue has formed (Slatter, 2003). In current study the extent of granulation tissue increased in all the treated groups form day 0 post-wounding till 7 except control group (on day 14) . On day 21 post-wounding only *salix* treated wounds showed areas with intact skin or partial to complete thickness. As healing proceeds, the newly formed granulation tissue which is red due to blood vessels fades with healing because the vessels disintegrate due to apoptosis (Slatter, 2003). Therefore, healing is better in *salix* treated wounds as the granulation tissue was complete as early as day 14 and then started fading and normal skin started appearing. This could be due to early completion of inflammatory phase in *salix* treated wounds and change to proliferative phase which constitute formation of new blood vessels and increased fibroblastic proliferation which together constitute granulation tissue (Slatter, 2003).

5.5.1.4. Extent of Cicatrization

Cicatrisation is the process of formation of scar. Matrix formation requires the removal of granulation tissue with revascularization. A framework of collagen and elastin fibers replaces the granulation tissue. This framework is then saturated with proteoglycans and glycoproteins. This is followed by tissue remodelling involving the synthesis of new collagen mediated by TGF-β. The final product of this process is scar tissue (Kondo and Ishida, 2010). In the current study, the salix treated wounds showed light pink, smaller and softened scar on day 7 post-wounding whereas the control group showed thicker and red wound areas. Towards the end of study period on 21, all wounds treated with *salix* showed fade thin to white line appearance of wound site, whereas in povidone iodine treated wounds showed softened scar formation and there is no clear cut scar formation in control group.

5.5.1.5. Extent of granulation tissue

The production of wound exudates is a normal part of the inflammatory process (Thomas, 1997). Fluid from acute wounds plays an essential part in healing process and the exudates from these wounds are rich in growth factors that promote tissue repair (Chen *et al.,* 1992). Histamine and serotonin released from the damaged cells increase capillary permeability, allowing plasma leakage from the blood vessels, which in turn leads to the accumulation of fluid in adjacent tissues (Cutting and White, 2002). In present study no measurable exudates was found in wounds treated with *salix* and povidone iodine except control group. Wounds have been shown to re-epithelialize more rapidly under moist conditions rather than dry conditions (Dyson *et al.,* 1992). Also autolysis, a natural degradation of devitalized tissue is most effective in a moist environment (Tong, 1999). Increased production of exudates may be associated with high bacterial growth in the wound. Wounds treated with povidone iodine as well as salix are reported to possess antibacterial activity (Lacey, 1979; Ali and Aboud, 2010). So, no infection was produced either in povidone iodine treated wounds or salix treated wounds. However, the wound bed remained moist allowing proper nutrition and healing process although healing was better in *salix* treated wounds due to its wound healing potential.

5.5.2. Morphometry of wound

5.5.2.1. Wound Size and Percentage Healing

In the present study though the original wound were created by measuring 1.5 x 1.5 cm area, almost all the wounds expanded to some extent possibly due to lose nature of skin on the dorsal thoracolumbar region of rabbits (i.e., flaccidity/pliability). Thus immediately after the surgery, all the wounds had an area greater that 2.25 cm^2. Therefore, the percentage of wound size (contraction) was calculated by measuring the actual size of wound on day '0' (not the predetermined area). Further, on day '0', difference in the mean wound area of all the treatment groups was statistically significant. As described by Kumar and Tyagi (1972), Bigbia *et al.* (1991) and Handoo *et al.* (2014), the tracing of the wounds on transparency sheet/cellophane paper and graph paper gave accurate data regarding the size of the wounds and it was found cheap, reliable and easy to calculate the wound size (length and width measurements) and percentage healing from the tracings. It involved no hi-tech methods and also it was simple to calculate wound area by adopting this technique. These observations substantiate with the findings of Thomas and Wysocki (1990), Griffin *et al.* (1993); Brown-Etris *et al.* (1994), Xakellis and Frantz (1997), and Bohling *et al.* (2004).

An accurate and thorough wound assessment is an essential component of optimal wound care (Bergstrom *et al.,* 1994; Copper, 1992; Marquez, 1995 and Thorman, 1997). A key parameter that should be included in a wound assessment is the measurement of wound extent (Lazarus *et al.,* 1994). Although each method of estimating wound size has inherent strengths and weaknesses, those used to determine wound surface area, rather than wound depth or volume, are believed to be more accurate and reliable (Mayovitz et al., 1998; Langemo et al., 1998; Plassmann, 1995; Thomas and Wysocki, 1990 and Xakellis and Frantz, 1997).

Gradual decrease in the size of the wounds and increase in percent as wound contraction was recorded in both the treated groups up to day 21 post-wounding. Reduction in the wound size was minimal in group NS. The wounds of *salix* treated groups evinced complete healing (100% contraction) which was followed closely by povidone iodine and distantly by NSS on day 21 post-wounding, where mean wound contraction/size of 100%. 95.66% and 61.00% respectively was recorded as observed by (Madhu, 2012)

Salix spp. have been reported to be used for antiseptic (Moore, 1979), hemostasis, and healing (Hart and Jafferey,1981, Moerman, 1998), antifungal agents (Malterud *et al.*,1985), treatment of rheumatic fever, colds, hemorrhages and goitre, and as a general antiseptic for wounds and abscesses (Ho *et al.,* 1985), wound healing agent (Elias , 1998). During this study wounds treated with salix showed better wound healing followed by povidone iodine and then the sterile normal saline solution. This could be attributed to anti-inflammatory, anti-rheumatic, antipyretic, antidotic, analgesic, and antiseptic properties (Barnes *et al.,* 2002) of *salix.* Wound healing mechanisms may be contributed to stimulate the production of antioxidants in wound site and provides a favorable environment for tissue healing (Shukla *et al.,* 1999) and wound healing effects may be due to up-regulation of human collagen I expression (Bonte *et al.,* 1993) and an increase in tensile strength of the wounds (Suguna *et al.,* 1996). Enhanced healing activity was attributed to increased collagen formation and angiogenesis (Shukla *et al.,* 1999; Trabucchi *et al.,* 1986). Angiogenesis in granulation tissues improves circulation to the wound site thus providing oxygen and essential nutrients for the healing process with enhanced epithelial cell proliferation (Szabo *et al.,* 1995). *Salix* has been reported to posses antioxidant and anticancer potential (Enayat, 2013), which could have further helped in healing process (Elias, 1998).

Wound contraction is the centripetal movement of wound edges resulting in diminution of the wound size (Hanks and Spodnick, 2005) and it depends upon the reparative abilities of the tissue, type and extent of the damaged general health state of the individual (Singh *et al.,* 2005). Contraction occurs simultaneously with granulation and epithelialization, but independent of epithelialization (Fossum *et al.,* 2007).

The percentage healing denoted the amount of wound contraction. Unlike epitheliazation, which closes the wound surface, contraction is a process that actually pulls the entire wound together, in effect shrinking the defect. Successful contraction results in a smaller wound to be repaired by scar formation (Hardy, 1989). Percentage healing varies as according to different observation intervals in each group significantly, depicting an increase in healing from start to the end of observation period. The extract of *salix* contain glycosides (1.5-11%) (Bissett, 1994; Mc Guffin *et al.,* 1997); salicylates (salicin, salicortin, populin, fragilin, tremulacin) (Meier *et al.,* 1985; Zaugg *et al.,* 1997); tannins (8-20%) (Thieme, 1968); aromatic aldehydes and acids: salidroside, vanillin, syringin, salicylic acid, caffeic and ferulic acids; salicyl alcohol (saligenin); flavonoids (Bissett, 1994; Mc Guffin *et al.,* 1997). Tannins are capable of precipitating proteins, resulting in shrinkage of cells. This precipitating protein forms a coagulum. Underneath the coagulum quicker regeneration of tissue takes place (Zama *et al.,* 1991). The percentage healing is more in wounds treated with *salix* followed by povidone iodine and then the sterile normal saline solution.

5.5.3. Bacterial colony count

Bacterial infection reduces wound contraction and consequently delays the wound healing process (Smith and Enquist, 1967 and Bucknall, 1980). In order to have optimum wound healing process, microbial load has to be kept on minimum possible level. The bacterial colony count decreased in all groups. In positive control group wounds treated with povidone iodine, has a good antibacterial action. These observations are in consonance with Lacey (1979) who reported use of povidone iodine in stimulated ulcer experiment and concluded povidone iodine as a potent and persistent bacterial activity and recommended its use in treatments of wound infection. Its bactericidal action has been reported against *K. pneumonia, P. cepacia* and *S. aureus* (Berkelman *et al.,* 1982). Zarger *et al.(*2014) stated that *salix* spps. have abundant watery bark sap, which is heavily charged with salicylic acid. Besides, salicin, it contains flavonoids and proanthocyanidins, which are potent wound healing agents. Flavenoids, besides having astringent and anti-microbial improves vascularity thereby enhancing wound contraction and increased rate of epithelialization. These findings match with those of Moerman (1998) who reported that the active ingredient of the *salix* bark is called salicin. Salicin hydrolyzes in aqueous media to glucose and salicylic alcohol (saligenin). The bark and leaves can be pounded and applied to wounds as healing agents. Phytochemical screening for *Salix acmophylla* reveals a moderate concentration of alkaloids, coumarines, cardiacglycosides, ratenges, phenols, flavonoids, saponins, tannins,essensial oil and terpenes some of which chemical compounds have been associated to antibacterial activities and thus have curative properties against pathogens (Nweze *et al.*, 2004). Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms (Doughari, 2006). These findings corroborate with that of Ali and Aboud (2010) who reported mature antimicrobial activity of *Salix acmophylla*.

5.5.4. Photographic evaluation

Wound size can also be estimated by photographic evaluation, by determining the maximum wound length and width using the ruler that had been placed next to the wound at the time of photograph was taken (Houghton *et al.*, 2000). But to achieve accurate clinical photographs of chronic wounds, exercising as much control as possible over variables that influence photographic results such as equipment, materials, film processing subjects, lighting and background is necessary. Also it requires trained healthcare professionals to achieve best photographs. Other determinants of wound healing that should be calculated in a wound assessment are the evaluation of wound bioburden and wound severity. This requires examining wound exudates and necrotic tissue type, the amount and characteristics of necrotic tissue, granulation tissue, and re-epitheliazation and assessing the viability of the wound edge and peri-ulcer skin (Bates-Jensen, 1995).

5.5.5. Histo-pathological observations

Histological analysis of healed wounds confirmed the gross observations. Healing of full thickness skin/excisional wound occurs by epithelialization and wound contraction. Epithelialization begins almost immediately (24 to 48 hours) in sutured wounds with good edge to edge apposition because there is no defect for granulation tissue to fill whereas in open wounds, epithelialization begins when an adequate granulation bed has formed (usually in 4 to 5 days) (Fossum *et al.,* 2007).

Healing of full-thickness skin wounds rely on granulation tissue formation, wound contraction and epitheliliaztion from the bordering skin (Gourley, 1978; Pope, 1998; Waldron and Zimmerman-Pope, 2003). Wound contraction is mediated by myofibroblasts (Fossum *et al.,* 2007) which have the features of both fibroblasts and of smooth muscle cells and contain alpha-actin, which play major role in wound contraction (Singer and Clark, 1999; Yamaguchi and Yoshikano, 2001).

During normal healing process following surgery or injury, there is swelling as one of cardinal signs of inflammation (Diegle-mann and Evans, 1981). Inflammatory mediators like histamine cause blood vessels to become porous, allowing the tissue to become edematous, because proteins from the blood stream leak in to the extravascular space which increases its osmoloar load and draws water into the area (Stadelmann *et al.,* 1998). Increased porosity of the blood vessels also facilitates the entry of inflammatory cells like leukocytes into the wound site from blood stream (Theoret, 2004). In the inflammatory stage the monocytes also enter the wound site. These monocytes are differentiated to macrophages, and recruited at the injured sites. Macrophages play an important role in augmenting the inflammatory response and tissue debridement (Konodo and Ishida, 2010). In present study the macrophages showed a decreasing trend in all the groups towards the end of observation period. However in control group the macrophage number remained higher on day 14 and 21 although comparatively lower in *salix* treated wounds group. Similar findings were observed by Handoo (2013) who reported that *Rhubarb* treated groups showed decreasing trend of macrophage score as compared to that of control group. Present study showed that the *salix* treated wounds showed better healing than control. This difference may be attributed due to the anti-inflammatory property of slaix (Barnes *et al.,* 2002). Once the inflammatory phase is over, the peripheral swelling disappears and reparative process initiates (Gourley and Vasseur, 1985).

The granulation tissue of the wound is primarily composed of fibroblasts, collagen fibers and small new blood vessels. The collagen is the major component of extracellular tissue, which gives support and strength (Singh *et al.,* 2005). Granulation tissue is important for the healing of open wounds because it is extremely resistant to infection and serves as a barrier against systemic infection, provides a surface over which epithelium is able to migrate, plays a role in wound contraction and contains the fibroblasts that produce the collagen for wound healing (Swaim and Henderson, 1990).

Abramo *et al.* (2004) suggested that histo-pathological assessment of mode and rate of healing in open wounds allows more precision that clinical and high resolution ultrasound, though it does not allow serial examination of wound sites. In the present study, however serial examination were possible as the biopsies were collected at different stage of wound healing. The biopsies were taken for each treatment on 7^{th}, 14^{th} and 21^{st} post-wounding days which helped in providing evaluation of wound healing as reported by Gupta *et al.* (1993). After routine histological processing biopsy samples were stained by H&E (Luna, 1986) to detect morphological changes and Mason's Trichrome stain (MTS) (Masson, 1929), to assess the collagen content of the healing wounds as collagen is a good marker for wound healing.

In the initial stage of repair (immature scar) than collagen fibers are seen, whereas thick collagen fibers are predominantly found in mature scar (Chandraratna *et al.,* 1997) and the orientation of the fibers is initially haphazard but lesser tension of wounds cause fibroblasts, fibers and capillaries to orient parallel to the incision or wound margin (Fossum *et al.,* 2007).

There was decrease in formation of exudates in all the treatment groups as the days passed. However, *salix* treated group resulted in faster reduction in wound exudates (serous type) and drying of the wound other than treatment groups by day 7, which was suggestive of accelerated wound healing (Tyagi and Singh, 1993; Ansari, 2014). This might be due to the presence of active agents in the respective treatment which has helped in the early enhancement of wound healing. The differences between the groups were significant and from day 14 onwards treatment groups showed similar results with apparent drying of the wounds.

Irrespective of the given, there was nil to moderate inflammation at the periphery of the wound of some of the animals on day 1 and day 2 while only in a few wounds by day 3 post-wounding, which subsided to nil on 7th day post-wounding. The slight swelling recorded in few cases may be considered as normal body response to trauma.

The granulation tissue as seen grossly appeared in significantly lesser duration in wound treated with *salix* than other treatment and control group. The findings were also supported by the histopathological findings of early maturation of granulation tissue in *salix* treated group. Regardless of the treatments used, granulation tissue was below the level of skin between 7 and 14 and proliferated to the level of skin edges and completely filled the blood bed by day 21 post-wounding in povidone iodine treated group whereas *salix* treated group granulation tissue was proliferated to the level of skin edges by 14th day post-wounding.

In the treatment groups, *salix* and povidone iodine treated the scab fell off the wound by 21 post-wounding day except NSS treated group. At this stage, the classical shinny, rose pink/pale red granulation tissue was seen indicating healthy healing progress as described by Kumar and Chauhan (2003), Hanks and Spondnick (2005), Fossum *et al.* (2007). The scar colour changed from rosy pink to paler in the later stage, which reflected the progression of blood vessels during maturation and remodeling as suggested by Waldron and Zimmerman-Pope (2003), Hanks and Spondnick (2005), Fossum *et al.* (2007).

By day 7th post-wounding, granulation tissue associated with admixture of mononuclear and polymorphonuclear cells was seen in *salix* treated wounds which was thicker than adjacent skin. The povidone iodine treated wounds showed mild to moderate inflammatory reaction with predominant heterophilic infiltration, underneath the scab, whereas wounds treated by NSS group showed necrosed tissue having pus cells underneath the scab.

By day 14 post-wounding *salix* treated wounds showed marked granulation tissue associated with vascularisation and heterophilic infiltration. The betadin treated wounds showed granulation tissue with fibroblasts proliferation varying moderate to predominant, whereas wounds treated by NS group showed severe heterophilic infiltration

By day 21 post-wounding, wounds treated with *salix,* showed no inflammatory reaction had denser and thicker collagen fibers in granulation tissue and are partial to complete epithelialization tendency to form hair follicle similar to normal skin. The povidone iodine treated wounds dense collagen deposition with partial epithelialization was observed, whereas wounds treated with NS showed predominant amount of collagen deposition associated with partial to complete but immature epithelialization. Angiogenesis, fibroplasias and epithelialization mark the proliferative and maturation phase of wound healing (Clark, 1996). The change from inflammatory to proliferative phase is marked by increased fibroblasts, increased accumulation of collagen in wound and increased angiogenesis. This change occurs at 3 to 5 days post wounding. Activated fibroblasts also migrate to the wound bed and along with macrophages from the granulation tissue (Slatter, 2003). In the present study the amount of fibroplasias and angiogenesis remained higher in wounds treated with *salix* group followed by povidone iodine and then control group on day 7 and 14 post-wounding. This is in accordance to the observation of Aakoon (2001) who reported marked proliferation of fibroblasts and neo-vascularisation on day 10 post-wounding. A massive angiogenesis allowing the supply of oxygen and nutrients is necessary for the healing process within the tissue (Singer and Clarke, 1999). The last stage of wound healing involves the gradual involution and regeneration of dermis. Collagen content is one of the necessary parameter for determining the pharmacological effects of potential wound healing agents (Jimenez and Rampy, 1990). In present study the collagen content of wounds treated with *salix* group remained highest throughout the observation period followed by wounds treated with povidone iodine than by control group. Epithelialization showed an increasing trend towards the end of observation period, this trend being highest in *salix* treated wounds than in povidone iodine treated wounds with control group showing least amount of epithelialization. These findings corroborate with that of Aakhoon *et al.* (2001) who reported mature collagen content in *salix* treated wounds on day 15 post-wounding. Histopatholigcally, epitheliazation, angiogenesis and fibroplasias level were higher in *salix* treated wounds on day 7, which indicates that *salix* accelerate the inflammatory reaction and initiate early phase of wound healing. Since the time of collect biopsy sample was fixed to maximum of 21 days in the experimental design, it was not possible to follow the wound healing beyond 21 days to record the day of compare healing in sterile normal saline (control group) treated wounds. However, proportions of wounds with complete healing were calculated at each interval for each treatment.

` There was significant difference in neovascularization of wounds treated by *salix* group followed by povidone iodine treated wounds as compared to control group. In conclusion, in the sterile normal saline treated wounds (control group), the epithelialization of the wound did not compare well with the normal skin, while in *salix* treated wounds it compared well with the normal skin followed by povidone iodine treated wounds. The current study indicates that the dressing with salix acmophylla leaves ointment, as topical application of wounds, enhanced wound healing process significantly in experimental rabbits.

Chapter – 6

SUMMARY AND CONCLUSIONS

In present study, four full thickness excisional skin wounds (1.5 cm × 1.5 cm), 2.5 cm apart from each other were created (dorsal spine of thoraco-lumbar region) on each of 18 rabbits (three groups having six animals) of either sex and weighing 1.8 to 2.5 kg under standard anesthetic protocol to evaluate the wound healing potential of *salix acmophylla* leaves ointment and their response on clinico-physiological, haemato-biochemical, kidney function test, bacterial viable count, gross and histomorphological parameters. The total number of wounds evaluated in the study was 72 and thus each treatment groups was evaluated on 24 wounds. The experimental study was conducted in three groups (I, II and III). Group I animals were treated with sterile normal saline solution (negative control). Groups II and III rats were dressed topically with thin layer of 5% povidone iodine ointment and 5% *salix acmophylla* leaves ointment, respectively. Poviodone iodine treated wounds were used as a positive control group. Rabbits were evaluated for change in rectal tempereature (RT), heart rate (HR), respiration rate (RR), total erythrocyte Count (TEC), differential leukocytic count (DLC), hemoglobin, packed cell volume (PCV), glucose, albumin, total protein (TP), creatinine level, blood urea nitrogen (BUN), wound size, percentage healig, total bacterial count level, photographic evaluation, gross and histomorpholigical examination.

The temperature (^{0}C) showed a non-significant (P>0.05) increase from day 1 post wounding in all animals of all the groups except significant increase on day 3 and 5 in group II and significant decrease on day 3 and 5 in group III as compared to base value. The value of RT (^{0}C) in the all the groups were within the normal range without showing any significant difference among them. However the highest values were recorded in the animals of group I as compared to groups II and III post wounding.

The HR (/min) showed a significant (P<0.05) increase on day 2, 7 and 14 post wounding in group I as compared to base value. The HR (beats/min.) showed a significant ($P<0.05$) increase from day 1 in group II as compared to base value. The HR showed a significant ($P<0.05$) increase from day 2 in group III as compared to base value. The value of heart rate HR in the all the groups were within the normal range without showing any significant difference among them. However the highest values were recorded in the animals of group II and III. The value of respiration rate, RR (breaths/min) in the all the groups were within the normal range without showing any **SIGNIFICANT ($P>0.05$),** difference in all groups as compared to base value and at different intervals. However the highest values were recorded in the animals of group II and III.

The decrease in TEC was significant ($P<0.05$) on day 3 and 7 in group I, on day 3, 7 and 14 in group II and on day 7, 14 and 21 in group III as compared to base value. The increase in Total Erythrocyte Count was significant ($P<0.05$) on day 21 in groups II and III as compared to base value. Total Erythrocyte Count did not show any significant ($P>0.05$), change in all groups at different intervals.

A significant ($P<0.05$) increase in Total Leucocytes count (TLC) was noticed from day 3 to 14 in group I as compared to base value. A significant ($P<0.05$) increase in TLC was noticed on day 3 to 7 and significant ($P<0.05$) decrease on day 14 and 21 in group II as compared to base value. A significant ($P<0.05$) increase in TLC was noticed from day 3 to 14 and significant ($P<0.05$) decrease on day 21 in group III as compared to base value. In comparison to group I, higher values of TLC were recorded in groups II and III, which were significant from day 3 to 21 higher in group III. At different intervals, neutrophil count showed an increasing trend in all the groups throughout the period of study except on day 21. The neutrophils increased significantly ($P<0.05$) from day 3 to 14 in groups I, II and III as compared to base value. On day 3, the neutrophil count was significantly ($P<0.05$) decrease in group II followed by group III as compared to group I and day 21, the neutrophil count was non-significantly ($P>0.05$) decrease in group III followed by group II as compared to group I. At different intervals, lymphocytes showed a decreasing trend in all the groups throughout the period of study except on day 21. The decrease in lymphocytes was significant (P<0.05) from day 3 to 14 in all groups except significant (P<0.05) increase in group III, on day 21 in groups III as compared to base value. On day 7 and 21, the value was significantly (P<0.05) higher in group III as compared to groups I and II. The increase in monocytes was significant (P<0.05) on day 3 and then there was significant (P<0.05), decreasing trend in all the groups as compared to base value. Monocytes did not show any significant (P>0.05), change in all groups at any different intervals. The value of basophils (%) in the all the groups were within the normal range without showing any significant (P>0.05), change in all groups as compared to base value and at different intervals. The value of eosinophils (%) in the all the groups were within the normal range without showing any significant (P>0.05), change in all groups as compared to base value and at different intervals.

In all the groups, the Hb decreased significantly (P<0.05) on day 3 as compared to base value. In comparison to group I, high levels of Hb was recorded in groups III followed by II and the values were significantly (P<0.05) higher in group group III followed by group II on day 21. In groups I and III, non-significant (P>0.05) decrease on day 7 and day 14 was recorded respectively as compared to base value. In group II, significant (P<0.05) increase value from day 3 to 21 day as compared to base value. However, the value of Hb in the all the groups were within normal physiological limits without showing any ***SIGNIFICANT (P>0.05), CHANGE*** in all groups at different intervals. In all the groups, the PCV increased significantly (P<0.05) on day 3 and 7 as compared to base value. However, the value of PCV in the all the groups were within normal physiological limits without showing any ***SIGNIFICANT (P>0.05), CHANGE*** in all groups at different intervals. The glucose showed a non-significant increase (P>0.05), from day 3 to 21 in group I and on day 14 and 21 in group III except on day 3 in group II, where the value was significantly lower (P<0.05) and on day 14 and 21 the value was significantly higher (P<0.05) from the base value. However, the value of glucose in the all the groups were within normal physiological limits without showing any significant (P>0.05), change in all groups at different intervals.

The Albumin showed a significant decrease (P<0.05), from day 3 to 14 in all the groups except on day 21 in group II, where the value was non-significantly higher (P>0.05) and on day 21 the value was significantly higher (P<0.05) in groups I and III as compared to the base value. However, the value of Albumin in the all the groups were within normal physiological limits without showing any significant (P>0.05), change in all groups at different intervals. The Albumin showed a significant decrease (P<0.05), from day 3 to 14 in all the groups except on day 21 in group II, where the value was non-significantly higher (P>0.05) and on day 21 the value was significantly higher (P<0.05) in groups I and III as compared to the base value. However, the value of albumin in the all the groups were within normal physiological limits without showing any significant (P>0.05), change in all groups at different intervals.

The creatinine showed a significant increase (P<0.05) from day 3 to 14 except non-significantly higher (P>0.05) value on day 21 in group I and in group II, where the value was significantly higher (P<0.05) on day 3 and 7 and significant (P>0.05) lower value on day 14 and 21 and the value was significantly higher (P<0.05) on day 3 and 7 and significantly lower (P<0.05) in group III as compared to the base value. However, the value of creatinine in the all the groups were within normal physiological limits showing significant (P<0.05) lower value change in II and III groups as compared to group I at different intervals except on day 21. The BUN showed a non-significant decrease (P>0.05) throughout the study period in group I except non-significantly higher (P>0.05) value on day 21 in group I and on day 3 and 7 in group II as compared to the base value. However, the value of BUN in the all the groups were within normal physiological limits showing significant (P<0.05) value in II and III groups as compared to group I on day 7.

A continuous progressive decrease in Total Bacterial count level was noticed in all the groups till day 21. The decrease from the base values was significant (P<0.05) in all groups from day 3 as compared to the base value. In comparison to group I, low levels of Total Bacterial count was recorded in groups II and III and the values were significantly (P<0.05) lower in group III followed by group II at different intervals.

Post wounding a significant (p<0.05) decreasing trend in the area of wound size was noted in all the groups on all observation intervals. The complete closure of cutaneous excisional wounds was noted by day 21 in the animals of group III. Comparison among the groups revealed significant (p<0.05) decrease in the wound size in the animals of treated groups (2 and 3) from those of control group (1) on all corresponding observation intervals. Furthermore the wound size value was significantly (p<0.05) lower in the animals of groups II and III from those of group I on all corresponding observation intervals from day 3 to 21 post wounding. Post wounding the percentage healing increased significantly (p<0.05) in all groups, at all observation interval with the result cent percent healing was achieved in the animals of group III by the end of the observation period. The healing was still incomplete in the animals of group I and II by the end of the observation period. Comparison among the group showed significant (p<0.05) increase in percentage healing of all treated wounds (groups II and III) as compared to control wounds (group I). Among treated groups percent wound healing was significantly (p<0.05) faster in the animals of group III as compared to the animals of group I and II.

Photographic evaluation of excisional wounds of rabbits was performed on 0, 3, 7th, 14th and 21st day after surgery. The salient observations of the photographic observation suggest that all the excisional wounds aseptically created were almost equi-dimensional on day ‘0’ (immediately after surgery).

Creation of excisional wounds resulted in variable extent of bleeding and formation of clot. The clot was dried and formed a cover over the wounds, which rendered the evaluation of colour of granulation tissue difficult. The scab, which covered the underlying granulation tissue, was detached in majority of the wounds after 14th post-wounding days. The scar became paler with passage of time, which indicated stage of maturation. Gradual decrease in the size of the wounds and increase in percent wound contraction (healing) was recorded in all the groups up to day 21 post-wounding. It was interesting to note on day 7 after post-wounding that there was appreciable reduction in size of wound treated with *salix* and povidone iodine as compared to control group/wounds. Furthermore, complete filling of the wound with granulation tissue without scab and demonstrable wound contraction was noticed in *salix* on day 14 post-wounding that povidone iodine and normal saline group. Complete epithelialization and closure of *salix* and povidone iodine treated wounds could be distinguished on day 21 and percentage healing 100.00% and 95.66% respectively were observed, whereas in NS complete healing was noticed in only 61.00% of wounds.

Grossly, wounds treated with 5%, *salix acmophylla* leaves ointment significantly accelerated the rate of wound healing compared to wounds treated with sterile normal saline solution or dressed with povidone iodine ointment. Histological analysis of healed wounds confirmed the gross observations. Wounds dressed with 5%, *salix acmophylla* leaves ointment showed markedly less scar width at the wound enclosure with large amounts of fibroblasts proliferation, more mature and densely packed collagen and angiogenesis compared to wounds dressed with sterile normal saline solution.

Overall results indicated that there was wounds dressed with *salix acmophylla* leaves ointment (group III) showed considerable signs of full thickness skin wound healing and significantly ($P<0.05$) healed earlier in 17.83 days followed by povidone iodine treated wounds (group II) in 19.00 days than wounds dressed with sterile normal saline solution (group I) in 20.83 days.

No physiological side effects and other complications were observed in any group. These results strongly document the beneficial and significant effects of *salix acmophylla* leaves ointment for the acceleration of wound healing enclosure in rabbits.

Conclusions

On the basis of results following conclusions can be drawn:

- Full thickness excisional wounds provide a better and easy way to observe different aspects of wound healing especially gross evaluation of wound.
- Locally fabricated metal marker (1.5 cm x 1.5 cm), 2.5cm distance apart provided an easy way to demarcate the margins of wounds to be created.
- Tracing of wound margins on cellophane paper provided easier method to calculate wound healing percent.
- Topically applied *salix* leaves ointment did not have any untoward effect on clinical and heamato-biochemical parameters.
- Present study suggested that topically applied *salix* leaves ointment showed no ill/adverse effect on kidney function test and could be effective for early wound healing.
- Topically applied *salix* leaves ointment induced faster and better wound healing as evidenced by early wound contraction, formation of granulation tissues, neovascularization, synthesis of collagen and histological maturation in excisional wounds indicating its activity as compared to NSS and povidone iodine treatments at the end of the study period. These results strongly document the beneficial and significant effects of *salix* leaves ointment for the acceleration of wound healing enclosure in rabbits.
- *Salix acmophylla* ointment can be used for the wound healing agents in field condition.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Prof. (Dr.) M.M.S. Zama, Professor, Dean, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Jammu, RS Pura during evaluation and Viva-Voce examination held on 30th of May, 2016 have been incorporated in the manuscript entitled, **"Wound healing potential of *Salix acmophylla* in full thickness skin wound using rabbit model"** submitted by **Dr. Zahid Rahim Malik (Regd. No.2013-V-250-M).**

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