STUDIES ON THE EFFICACY OF SEABUCKTHORN (*Hippophae rhamnoides*) IN THE HEALING OF CUTANEOUS WOUNDS IN DOGS

THESIS

BY

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Submitted to

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IN

Partial fulfillment of the requirements for the degree

OF

MASTER OF VETERINARY SCIENCE
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Borrower's No.	Due Date	Borrower's No.	Due Date

Is there anything I can say?
Anything I can give
Or do for you
Because all that I am
All that I have
I owe to you

Affectionately dedicated to my beloved Parents

Dr. M. S. Kanwar
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CERTIFICATE I

This is to certify that the thesis entitled **"Studies on the efficacy of seabuckthorn*(Hippophae rhamnoides)* in the healing of cutaneous wounds in dogs"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the subject of **Veterinary Surgery and Radiology** of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, is a *bonafide* research work carried out by **Dr. Munish Gupta** son of Shri Kulbhushan Gupta under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation has been fully acknowledged.

M S Kanwar

Dr. M.S. Kanwar
(Chairman)
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Place: Palampur
Dated: 13.06.2002

CERTIFICATE II

This is to certify that the thesis entitled **"Studies on the efficacy of seabuckthorn*(Hippophae rhamnoides)* in the healing of cutaneous wounds in dogs"** submitted by **Dr. Munish Gupta** son of Shri Kulbhushan Gupta to the Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, (H.P.) . India, in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Sciences** in the subject of **Veterinary Surgery and Radiology** has been approved by the Advisory committee after an oral examination of the student in collaboration with an External Examiner.

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(MUNISH GUPTA)

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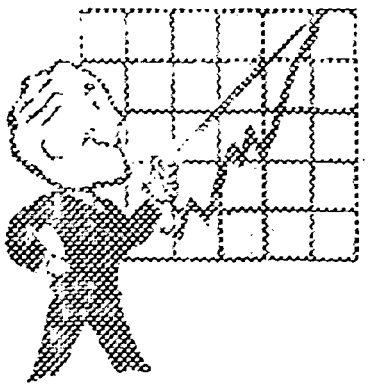
ABBREVIATIONS

SBT	:	SEABUCKTHORN
BET	:	BETADINE
LPF	:	LIQUID PARAFFIN
DPT	:	DAY POST TREATMENT
gm	:	GRAM
Hb	:	HAEMOGLOBIN
PCV	:	PACKED CELL VOLUME
TLC	:	TOTAL LEUCOCYTE COUNT
TEC	:	TOTAL ERYTHROCYTE COUNT
DLC	:	DIFFERENTIAL LEUCOCYTE COUNT
Sq. cm.	:	SQUARE CENTIMETER
Min	:	*MINUTE*
n	:	NUMBER
I/M	:	INTRAMUSCULAR
Kg	:	KILOGRAM
ml	:	MILLI -LITRE
cu.mm:	:	CUBIC MILLIMETER

INTRODUCTION



INTRODUCTION

Wound healing is a vital body response and always gets high priority. Even after tremendous advancement in the management of wounds, an effective wound management is a challenge to the clinician. In an ideally suited environment, the process of wound healing may be hastened due to the optimal biological response of the body. In order to create similar situations, application of different medicaments is suggested.

The use of medicinal plants in animal health is probably extremely important (Lambert *et al.*, 1997). Seabuckthorn plant (*Hippophae rhamnoides* family *Elaeagnaceae*) is renowned for its versatile pharmacological applications due to the presence of many known and unknown bioactive substances mainly in its fruits and leaves. Seabuckthorn is thorny, nitrogen-fixing, deciduous shrub or a small tree (2-5m). This plant is predominantly found in high altitude areas of different countries of Europe and Asia including India. In India, it grows wildly in high altitude areas of Himachal Pradesh, Jammu Kashmir and Uttar Pradesh. In Himachal Pradesh, this plant is locally named as 'chharma' and grows wildly on the river sides and sun facing slopes in Lahaul Spiti, parts of Chamba, Kinnaur, Kullu, Shimla and Kangra districts.

The utilization of seabuckthorn in medicine was first initiated by Chinese people as early as in the 8th century. The different parts of seabuckthorn have been used for the treatment of skin wounds and various ailments of cardiopulmonary and gastrointestinal system (Xioping *et al.*, 1995).

The different preparations of seabuckthorn such as decoction, powder, pill, medicinal extract, shortbread, ash and tincture has been used for the treatment of various disease conditions (Mingyu *et al.*,2001).

The therapeutic value of seabuckthorn oil has been reported in prevention of atherosclerosis (Olziikhutag, 1969) and enhancing the wound healing of superficial skin burns in rabbits (Vlasov, 1970). Russian scientists not only could be able to identify a number of key bioactive substances present in seabuckthorn but also substantiated their pharmacological actions through systematic research on experimental animals and also through many clinical trials on human beings as well (Mironov *et al.*,1989). The use of Seabuckthorn oil for the treatment of burns, skin radiation, cervical erosions, gastric and duodenal ulcers etc.has also been reported (Alieva, 1976). Biochemical and pharmacological studies on seabuckthorn were conducted in China mainly in last two decades.

A number of steroids, flavonoids and vitamins (particularly Vit. E & K.) found in seabuckthorn were thought to be responsible for its versatile pharmacological activities such as anti-inflammatory (Zhang-Wenlu *et al.*,1988), chemical or physical burn wound healing ability(Nikulin *et al.*,1992), anti-gastroulcerative activity (Xiao *et al.*, 1992), hepatoprotective (Cheng, 1990, 1992), anti-cancerous(Li and Liu,1991), anti-lipemic and anti-arrhythmic (Fengming *et al.*,1989) properties.

Armed with these findings now China and Russia are forerunners in exploiting the potential of seabuckthorn as a medicinal plant. There this plant is

being cultivated as a cash crop on lakhs of hectares of land. A number of pharmaceutical companies in these countries are making and marketing a number of seabuckthorn based products for treatment and prevention of a variety of ailments.

Taking leads from these developments, now over 40 countries of world are actively engaged in conducting research on this plant and harvesting its potential. In India, extensive field studies have been carried out to identify the normal habitat of this plant. The chemical composition of one of the Indian variety of seabuckthorn (*Hippophae salicifolia*) has also been studied (Ambaye and Indap, 1970). Indian Himalayas has the 2nd or 3rd richest resources of seabuckthorn (30,000-40,000ha) of the world. Lahaul Spiti in Himachal Pradesh and Nubra valley in Laddakh (J&K) has very rich resources of seabuckthorn. The three species of seabuckthorn have been identified in Himachal Pradesh so far (*H. rhamnoides, H. salicifolia and H. tibetana*) in which *H. rhamnoides* is predominant and therefore is widely used by amchies.

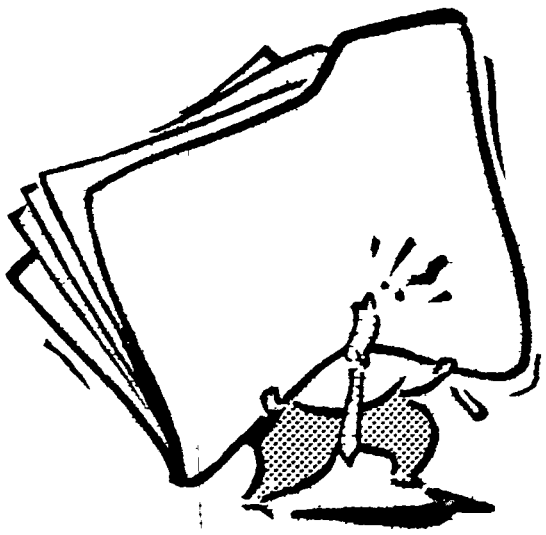
The fruits of this plant are widely used by the amchies (traditional medical practitioners) in these areas for treatment of different kinds of ailments like stomach disorders, lung disorders, skin diseases (Lal *et al.*, 2001). Most of the research work done in India on various medicinal aspects of seabuckthorn has been conducted by procuring the raw material from abroad and no work on Indian variety of seabuckthorn has been initiated so far. Efforts are being made all over the world to identify precisely newer agents that can improve the impaired healing process of soft tissues.

Keeping in view its ecological, pharmacological and in turn economical potential, active research is being done on this plant for its taxonomy, biochemistry, agroforestry and propagation in India but only very few studies have been undertaken to verify its medicinal applicabil ities in soft tissue repair (Gupta *et al.*,2001). Therefore the present study will be conducted to evaluate the efficacy of Indian variety of seabuckthorn fruits with reference to its most exploited actions on soft tissue repair particularly in cutaneous wound healing with the following objectives:

1. To evaluate the efficacy of seabuckthorn ointment in full thickness cutaneous wounds by clinical and gross examination in dogs.

2. To study the healing process of full thickness cutaneous wounds by histopathological and histochemical methods in dogs.

REVIEW OF LITERATURE



REVIEW OF LITERATURE

Wound healing has been studied with different angles relevant to different disciplines. The review of literature pertinent to the problem has been classified under the following heads:

2.1. Wound repair and effects of different medicaments on wound healing.

2.2. Therapeutic properties of seabuckthorn in wound healing and other disease conditions.

2.3. Therapeutic properties of various drugs/ biological materials on wound healing.

2.1. Wound repair and effects of different medicaments on wound healing:

Cutaneous wound repair is characterized by three overlapping phases involving inflammation, proliferation and remodeling. Various cellular reactions following trauma under normal environment have been studied and reviewed by Peacock (1962), Goldin and Joseph (1968), Ross (1969, 1971) and Heughan and Hunt (1975). After injury, new tissue formation starts with re-epithelialization and is followed by granulation tissue formation. The latter process encompasses macrophage accumulation, fibroblast in growth, matrix deposition and angiogenesis (Clark, 1991). These steps are orchestrated in a

controlled manner by a variety of bioactive molecules like growth factors, cytokines, their receptors and matrix molecules. Such a controlled phenomenon can be disrupted in diseases like diabetes, immunosuppression, aging and ischaemia etc.thus leading to the development of chronic wound. Prolonged or incomplete wound healing is then a troublesome complication (Ingold, 1993).

Tenorio *et al.*, (1976) described vigorous inflammatory response with increased wound strength (not correlated to wound collagen contents) in laparotomy wounds of rats and further observed accelerated wound healing due to an optimal inflammatory response by certain bacteria. A severe acute inflammatory reaction, more rapid formation of granulation tissue and epithelialization has been reported in conventional rats as compared to germ free rats (Donati *et al.*, 1971). The studies showed that normovolemic anaemia, unrelated to malnutrition, cancer or chronic infection does not appear to affect wound healing in experimental animals. (Heughan *et al.*, 1974 and Peacock, 1984).

Taylor *et al.* (1986) reported the effect of haemorrhages on wound healing in rats and found that the injections of 50mg/kg.of 1-ethoxysilatrane for three weeks accelerated wound healing in both unbled and bled animals. The impaired wound healing with mild to moderate, short or long term energy-protein malnutrition has been indicated in human patients although it is not proven in horses (Haydock and Grahan, 1986). Despite the benefits in preventing infection, topical antibiotics can retard wound healing. Generally, water soluble antibiotic solutions have less effect on wound healing than ointments or creams. Lee *et al.*

(1984) found gentamicin cream to delay epithelialization and wound contraction when used in the treatment of full thickness skin wounds in dogs, as compared to gentamicin solution.

There are reports that the ointments containing neomycin, polymixin and bacitracin increased the rate of epithelialization while nitrofurazone in a soluble base retarded wound healing by 24% (Geronemus *et al.*, 1979; Lee *et al.*, 1986). In in-vitro studies on povidone iodine solution, the dose dependent inhibition of neutrophil migration, monocyte function and fibroblasticidal effect has been documented (Tvedten and Till, 1985). A reduction in the major structural macromolecules, collagen formation and glycosaminoglycans were noted by using lidocaine and bupivacaine on tissue cultures of new born rat skin (Morris and Tracy, 1977). It has been found that insulin applied to wounds increases protein synthesis, cellular multiplication, wound contraction and fat deposition. It also enhances phagocytosis and reduces tissue edema (Swaim and Lee, 1987).

Lee *et al.* (1987) found occlusive or semi occlusive non adherent dressings to be most beneficial when applied to wound during the repair phase since latter enhances epithelialization and wound contraction. The use of magnetic dressings in the treatment of equine soft tissue injuries has become popular in Europe. The effects of an energy-Pak dressing delivering 400 Gauss with a maximum penetration depth of 7mm on skin wound healing in rats have also been evaluated (Leaper *et al.*, 1985). It is indicated that wounds created over the dorsal aspect of fetlock in ponies were best managed by leaving them

uncovered (Fretz *et al.*, 1983). The effects of isobutyl-2-cyanocrylate on skin healing in rabbits have also been described (Hampel *et al.*, 1991).

Barber (1990) studied second intention wound healing and the effect of bandages and topical corticosteroids on wound healing in the horse. Sanchez *et al.* (1988) reported the effects of different dilutions of chlorhexidine diacetate and povidone iodine on wound healing in dogs. He found more wound healed area and more contraction rates on days 7 and 14 than the saline treated wounds. The tripple antibiotic ointment containing polymixin-b sulphate, bacitracin, neomycin sulphate and an aloe-vera extract gel has been used for open wound healing of pad wounds in 15 Beagle dogs created under anaesthesia. The primary difference between the two medications was noticed at 7 days when the aloe treated wounds had a smaller unhealed area than did untreated control wounds and wounds treated with antibiotics (Swaim *et al.*, 1992).

The effect of chitosan oligomer on wound healing has been evaluated in dogs (Minami *et al.*, 1995) and he found it more effective on canine abscesses produced by inoculation of *staphylococcus aureus* than was treatment with antibiotics. Cornell and Waters (1995) studied impaired wound healing in human cancer patients and effect of cytotoxic therapy and pharmalogical modulation by growth factors. The role of epithelialization in wound healing has also been described (Fitch and Swaim, 1995).

Scardino *et al.* (1999) reported the effects of omega-3 fatty acid diet enrichment on wound healing in 12 Beagle dogs and found no outstanding long term negative effects on wound healing.

2.2 Therapeutic properties of seabuckthorn in wound healing and other disease conditions:

The ripe fruit of seabuckthorn is considered as a medicinal fruit. Its taste is sour as organic acid comprises 2-3 %(percent) of its weight. In former USSR it was discovered that fruits of seabuckthorn contained more than 190 kinds of bioactive substances and oil contained 106 kinds of such substances, of these there were 6 kinds of fat soluble vitamines,22 kinds of fatty acids, 42 kinds of lipids and 36 kinds of flavonoids and phenols (Zhang,1990). The versatile pharmacological activities of seabuckthorn oil were hence attributed to these bioactive substances. The chromatographic analysis of ripe fruit of seabuckthorn revealed the presence of malic acid, oxalic acid and other unidentified acids in it. These organic acids are known for certain physiological functions in body such as reducing the toxic effects of some medicines like barbitals and antibiotics, preventing teratogenesis, damages from the X- rays and side effects of oxygen therapy.

There are also significant contents of carotenoids (including Beta-carotene, Beta- 4, 4 Biketone- beta carotene, Gamma carotene, Zeaxanthin, Lycopene and Polyring- lycopene), progesin, flavoxanthin, cryptoxanthin, violaxanthin, neoxanthin and V_C, V_K V_E, (Vitamins) of which V_C and V_E are the major components of antioxidants(Qibikeva,1989). All these bioactive substances contained in seabuckthorn fruit and seed are responsible for its versatile therapeutic properties.

2.2 A: Role of seabuckthorn in wound healing:

Olziikhutag (1969) and Vlasov (1970) were among the first group of scientists who reported medicinal values of seabuckthorn oil against development of experimental atherosclerosis in rabbits and wound healing effects on superficial burns of skin respectively. Mironov *et al.*(1989) described repairative effects of seabuckthorn oil on rabbits and shown that complete healing of skin excision wounds can be attained with in 13-14 days as against 19-21 days in control animals. Buhatel *et al.* (1991) combined seabuckthorn oil with a large spectrum antibiotic and some other ingredients and showed favourable effects on the process of cicatrization of wounds in animals.

Khirurgiia (1995) studied the effect of an extract of seabuckthorn (H. *rhamnoides L*) on healing of experimental skin wounds in rats and found excellent results. Fayman (1991) reported treatment of operative wounds of ear, nose and throat with seabuckthorn oil. Nikulin *et al.* (1992) described the effects of seabuckthorn oil on chemical and physical burn wounds in rabbits. Gupta *et al.* (2001) reported very good healing potential of seabuckthorn seed oil and flavonoids on excisional cutaneous wounds in albino rats.

2.2 B: Role of seabuckthorn in other ailments:

Ulcer healing ability:

Fushun *et al.*(1989), Mingyu and Huai(1989), Guoli and Zhong(1989) recorded ulcer healing activities of seabuckthorn oil and attributed it to the presence of different flavonoids, terpenoids and some stress reducing compounds.

Jiang *et al.* (1989) noted that healing effects of seabuckthorn seed oil on white rats gastric ulcer model caused by acidification and chronic reserpinization were superior to cimetidine. Xu Hanqing *et al.* (1993) treated chronic skin ulcers with seabuckthorn oil by oral or external application and found improved curative effects.

Cardiovascular disorders:

Zhang Maoshun *et al.* (1987) treated coronary heart disease with TFH (Total flavonoids of Hippophae) and showed that TFH could remit angina and improves the mechano-cardiography and ischaemic electrocardiogram.

Wang *et al.* (1993) reported TFH to be effective in cardiac arrhythmias in white rats.

Immunomodulatory effect:

Seabuckthorn has got immunomodulatory effect. Renlisa *et al.* (1992) showed seabuckthorn seed oil to restore the natural killer cells under uninhibited state of immune function in mice with bone marrow micronucleus technique. Li, Diandong *et al.* (1993) concluded that multiplication index of spleenic lymphocytes of the mice who were fed seabuckthorn juice was higher than that of the control group at same age.

Anti-tumour effect:

Zhang *et al.* (1989) found that both intra-peritoneal injection of seabuckthorn oil and oral administration inhibited the tumour (sarcoma and lymphatic leukemia) in mice. Yang *et al.* (1989) conducted experimental in vitro

studies on mice and found inhibiting effect on the Elli's ascites carcinoma with seabuckthorn oil and its fruit residual.

Anti- senilism effect:

It is believed that senility and many lesions are closely related to peroxide effect in vitro. Therefore blocking the peroxidation and elimination of free radicals produced by the peroxidation have become the focuses of attention. JingYuehua *et al.* (1989) discovered superoxide dismutase in seabuckthorn juice and its leaves and concluded that its action was similar to that of vitamin C with antioxidation effects and clearing away the free radicals on cellular membrane.

Ju Haisong *et al.*, (1989) observed that TFH (Total flavonoids of Hippophae) could significantly inhibit the chemi-luminiscence of human polymorphonuclear leucocytes and clear away the superoxide free radical produced by the purine oxidase system thus delaying the aging process.

Anti-inflammation and Anti-radiation effects:

Xu Mingyu *et al.* (1993) described the role of seabuckthorn oil in eliminating inflammation and slough, easing pain, promoting immune function and strengthen body résistance. Lebedeva *et al.* (1989) evaluated experimentally the seabuckthorn oil in artificially induced inflammation of mouse subcutaneous tissue and found its anti-inflammatory effects. Wu *et al.* (1992) studied seabuckthorn oil and oil embolus with seabuckthorn compounds for the treatment of chronic cervicitis and showed 97 percent general curative effectiveness. Che Xiping *et al.* (1992) found therapeutic effects of seabuckthorn oil embolus on easing pain and eliminating inflammation.

Li Mingzhong *et al.* (1989) reported anti-radiation effects of seabuckthorn oil and other Chinese medicine mixture. Fan Yulin *et al.* (1991) used seabuckthorn oil in clinical treatment of traumatic perforation of tympanic membrane and reported full layer hyperplastic reunion of tympanic membrane. Fayman (1991) treated postoperative wound of tonsillitis with seabuckthorn oil.

Antibacterial and antiviral properties:

Seabuckthorn oil has also been shown to possess antiviral (Hiporamin) and antibacterial activities (Shipulina, 2001).

2.2C: Miscellaneous Properties:

Toxicity:

Rachimov *et al.* (1989) studied the acute and chronic toxicity of seabuckthorn oil by per os injection to mice, rats, cats and dogs. The injected doses exceed the therapeutic ones 20- 30 times. The studies of acute and chronic toxicity showed that the seabuckthorn oil does not influence the central and vegetative nervous system and practically is not a toxic substance.

Gupta *et al.* (2001) reported the effects of experimental feeding of Indian seabuckthorn (Hippophae-L) fruits in albino mice and observed its toxic effects when fed beyond 40% (percent) to albino mice, characterized by decrease in TLC and increase in bilirubin and ALT contents. 100 % (percent) feeding of seabuckthorn proved fatal to these albino mice as all mice died in the 3rd week of the experiment.

2.3 Therapeutic properties of various drugs/ biological materials in wound healing:

Varshney *et al.* (1994) evaluated the efficacy of saliva on healing of full thickness cutaneous wounds of male cow calves at different intervals till 28 days and found complete healing of wound cavities with granulation tissue by 16-20 days in saliva treated wounds. The medicinal value of neem oil (*Azadirachta indica juss*) as a potent wound healer has been established histomorphologically and histochemically on incised wounds in calves(Bhardwaj and Sharma,1997) which revealed faster and stronger union of incised and gap wounds with neem ointment. The faster wound healing properties of Himax and Teeburb in crossbred male jersindhi calves on incised lacerated wounds when used in combination have been reported (Sharma and Bhardwaj, 1995).

Few indigenous oils(Cedrus oil, Deodar oil ,Castor oil etc.) have been shown to be good promoters of wound healing with appreciable results (Bhargava *et al.*,1989).The detailed study on wound healing in camels with neem oil, protamine zinc insulin and camel tissue extract as topical medicaments has been evaluated for wound healing (Purohit *et al.*,1992). The role of charmil in the management of full thickness burns in buffaloes have been reported (Parikh *et al.*, 1996) and found complete healing of all treated wounds at 32 ± 2.60 days. Kumar *et al.* (1996) studied the clinical efficacy of charmil gel for wounds and skin affections in equines and indicated effective wound healing, antiseptic and fly repellent properties.

Shah and Dave (2001) studied the clinical efficacy of Frewund in treatment of all types of wounds in different species of animals and found good results. The evaluation of free full thickness. split thickness and pinch skin grafts in dogs has been carried out successfully with 41.66, 50.00 and 75% (percent) success rates respectively (Wani and Kulkarni, 1995). Pinch grafting for the treatment of an extensive wound of extremity in a dog has also been used (Vishwasrao and Mantri, 1990). The full thickness mesh grafting in septic wounds showing full thickness skin loss in a Labrador retriever dog has also been reported with very good results (Pattnaik and Parvathamma, 1999). The experimental studies have been conducted by Varshney *et al.* (1990) on the repair of abdominal wall defects by biological grafts in buffaloes and concluded that grafts were well accepted for repair of ventral abdominal defects. Parikh *et al.* (1996) performed the normal saline preserved auto skin grafting in buffalo calves and suggested that pinch skin grafts preserved in normal saline at 4^0 C up to 4 weeks can be used as auto grafts The role of occlusive dressings- Granuflex and Kaltostat on moist wound healing in dogs with significant healing of soft tissue has been reported (Adamiak, 2001).

Dehghani and Abrishami (1993) studied the effect of phenytoin on deep and superficial wound healing in rabbits and concluded that phenytoin is a safe drug and can be used effectively in the treatment of superficial and deep wounds. Kumar *et al.* (1997) reported the use of ethno-veterinary phytomedicines used in India and Nepal in treatment of fractures, wounds and allied disorders. Udasi *et al.* (1996) evaluated the efficacy of different concentration of urea

solution in the treatment of wounds and concluded that higher concentrations of 18 %(percent) hinders the healing process whereas 8% or 12% solutions favour rapid wound healing. The influence of anabolic steroid on biochemical constituents of granulation tissue in calves have also been reported (Nair *et al.*, 1998).

Ansari *et al.* (1997) described the effects of *Calendula officinalis* ointment, charmil and gelatin granules on wound healing in buffaloes histologically and concluded early and complete wound healing. Varshney and Verma (1990) reported efficacy of Himax in wound healing in bovines clinically and described it as a safe and dependable ointment with accelerated wound healing properties in animals. Varshney *et al.* (1997) histomorphologically studied the therapeutic value of bovine saliva in wound healing in male calves of 8-16 months of age and found very good healing rates and suggested its use for cutaneous wound healing in bovines.

Gupta *et al.* (1992) evaluated therapeutic efficacy of honey in infected wounds in buffaloes and reported faster epithelialization in honey applied wounds than those applied with other medicaments. The comparative studies on *adhatoda vasica* and pancreatic tissue extract on wound healing have been conducted in buffaloes which revealed high rates of healing in the former treated wounds followed by latter (Zama *et al.*,1993) The efficacy of swellnil in healing of experimental wounds in buffalo calves has been evaluated and found it to be very useful ointment in dressing of wounds as compared to zinc oxide ointment (Dabas *et al.*,1995).The clinical efficacy of multiaction skin gel

Charmil has also been described particularly in the cases of abrasions, cuts, gored wounds and surgical wounds (Rajaraman and Rao,1995).

Pradhan (1995) studied experimentally the effect of charmil on wound healing in bovine calves and found it useful in the treatment of suppurative wounds and maggot infested wounds of cattle. Prakash (1999) described the role of homeopathy drugs particularly *Calendula* preparations in wound healing and ulcer healing in animals. Agarwal (1997) noticed the therapeutic efficacy of an herbal gel for skin affections in dogs with significant results. Jadon *et al.* (1985) studied the effect of amnion on wound healing in buffalo calves and revealed early healing of wounds with amnion in comparison to control wounds.

Bandy *et al.* (1989) reported that floral honey and honey from the sugar- fed bees has a very good healing effect on surgical wounds in animals. Sumano-Lopez *et al.* (1989) established that Propolis essential oil (PEO) and aloe vera gel (AVG) mixed in ratio of 1:10 have superior healing properties compared to those of PEO and AVG alone. The comparative experimental studies on healing of skin, muscle and intestine wounds following scalpal and electrosurgery have been described in rabbits (Bakhtyari *et al.*, 1994).

A retrospective analysis on complications of wound healing in dogs and cats has been conducted (Brunnberg *et al.*, 1997). The epidemiological analysis of postoperative wound infections in dogs and cats has been reported (Brown *et al.*, 1997). The clinical and histological comparison of tissue damage and healing following incision with the carbon dioxide laser and stainless steel

surgical blade in dogs have been carried out (Durante and Kriek, 1993).The complete evaluation of multipeptide copper complex medications on open wound healing in dogs have been studied (Swaim, 1989).The complete wound healing occured in 7 days as compared to control groups. Nakade,*et al.* (1996) described accelerated effects of a natural polysaccharide, beta chitin on wound healing in dogs.

Similarly, Okamoto *et al.* (1995) evaluated chitin and chitosan on open wound healing in dogs and found good results. Arican and Ozturk (1999) conducted a clinical study using collagenase in the treatment of open or infected wounds with significant results. Saikia *et al.* (1998) described the role of placentrix in wound healing in dogs and cattle and found accelerated wound healing than in control wounds. Iwasaki *et al.* (1996) reported expression of the basement membrane macromolecules and integrin receptors by keratinocytes during wound healing in dogs. Mooney *et al.* (1998) evaluated the effects of Omega-3 fatty acids containing diets on the inflammatory stage of wound healing in dogs.

Scardino *et al.* (1998) evaluated the therapeutic effects of pulsed electromagnetic field on wound healing, clinicopathological variables and central nervous system activity in dogs. Swaim *et al.* (2000) studied the effect of a hydrolyzed collagen dressing on the healing of open wounds in dogs. The efficacy of local wound antiseptics (Lavasept and Braunol) for the treatment of wound healing by second intension has been described in dogs (Lierz *et al.*, 2000).

Successful treatment of wound on the mammae of a Labrador bitch and maggot wound on the thigh of a Boxer with chitin combined with conventional therapy has been reported (Sriram *et al.*, 2000). Chen *et al.* (1998) reported the influence of frequency spectrum apparatus irradiation on wound healing and blood in dogs. Tugnaiyat *et al.* (2000) described the efficacy of *Curcuma longa* and *Hellianthus annus* on tissue repair in calves.

Shrivastava *et al.* (2000) noted biochemical alterations in healing tissues with herbal preparations in cow calves. The effects of chlorhexidine diacetate, povidone iodine and polyhydroxyline on wound healing have been studied (Lee *et al.*, 1988) and suggested that 0.5% chlorhexidine should not be used as a wound antiseptic for prolonged period of time on the tissues. Tripathy (1990) reported the use of Himax- D lotion in the treatment of different skin troubles in dogs and recorded the excellent healing effects of Himax- D lotion once daily in septic punctured wounds.

MATERIAL AND METHODS

MATERIALS AND METHODS:

3.1 Experimental design:

The present study was conducted in nine adult healthy mongrel dogs of either sex (6-females and 3 males) weighing between 10kg-18kg. For this study, these dogs were divided into three groups of three animals each as per the following schedule:

Group - 1 **Seabuckthorn group (SBT)**

Group - II **Betadine group (BET)**

Group - III **Liquid paraffin group (LPF) - control group**

In each group, prior to the start of experiments. Butox spray @ (1 ml in 1L of leukwarm water) and bath of the animals was carried out 2-3 times in a period of 15 days during which the animals were kept in the college kennel so that they may get acclimatized to the new environment. The kennels were cleaned and fumigated regularly. The deworming of all animals in each group was carried out with albendazole @ 10mg/kg body wt. orally, single dose). The animals were also vaccinated prophylactically against rabies (Raksharab=1 ml s/c).

All the animals were maintained on adlib diet comprising of Bread/Chapaties, eggs, Dalia, Milk etc. and water during the entire course of the study.

3.2 Creation of the excisional cutaneous wounds:

Each animal in the present study was prepared aseptically before the creation of excisional cutaneous wounds. The dorsal surface of the thoracolumbar area was thoroughly washed with savlon and water, shaving was performed on the entire dorsal surface of the thoracolumbar area, scrubbed thoroughly using savlon solution, dried and applied with 70% alchohlic solution. In all the dogs of each group, equidimentional six full thickness excisional cutaneous wounds of dimensions 1.5 X 1.5 cm., 3 cm. apart were created with three wounds on either side of the vertebral column on dorsal aspect of the thoracolumbar region aseptically under general anaesthesia(Xylazine@2mg/kg and Ketamine @10mg/kg in a single syringe intramuscularly).

3.3 Therapeutic regimen:

The experimental animals were divided in to three groups. as described above.

Group – I:

The animals in this group were treated by topical application of seabuckthorn ointment.

Group- II:

The animals of group-II were treated by topical application of 5% povidone iodine ointment and served as positive control.

Group – III:

The animals constituting this group were treated by topical application of liquid paraffin (Negative Control).

In all the three experimental groups, comprising three animals each, the treatment by using three different therapeutic regimens was started from day '0' till 28th day post treatment (DPT). During the entire period of study, daily dressing of excisional cutaneous wounds was carried out with respective ointments and wounds were covered with sterilized gauge which in turn again covered by sterilized drapes. To prevent licking and biting at the wound site by the animals, the animals were restrained by using neck collars and muzzle piece.

3.4 Preparation of seabuckthorn ointment:

Seabuckthorn ointment was prepared from the sun dried fruits which were grinded in a cyclotone mill to form fine powder. The 50 gm of the grinded seabuckthorn powder was then mixed with 100 ml of liquid paraffin to form 50% seabuckthorn ointment. All these animals were then evaluated on the basis of: clinical, haematological, histochemical and histopathological studies.

3.5 Parameters studied:

1. Clinical observations:

Clinical observations such as rectal temperature, respiration rate, heart rate, degree of inflammation, appearance of wound exudation, extent of cicatrization and epithelialization and percent wound contraction were recorded at 0 and 3rd,7th,14th,21st and 28th day post-treatment. The degree of inflammation was categorized as no (-), mild (+), moderate (++), and severe (+++). The appearance of wound exudation was designated as no (-), mild (+), moderate (++), and severe (+++). The extent of epithelialization was also categorized as no

(-). mild (+), moderate (++), and extensive (+++). The percent wound contraction was measured by using the following formula:

Percent wound contraction= B-A÷B X 100

Where B is the area (sq. cm.)of wound at day 0 and A is the area (sq. cm.) of wound at day 3rd, 7^{th},14^{th}, 21^{st} and 28^{th} respectively.

2. Haematological studies:

About 2ml of venous blood was collected on days 0 and 3^{rd},7^{th},14^{th},21^{st} and 28^{th} day postoperatively from cephalic vein of all the dogs in each group in heparinized syringes and was analysed immediately for estimation of haemoglobin (Sahli's method), packed cell volume (microhaematocrit method), total erythrocyte count, total leucocyte count (neubaur chamber) and differential leucocyte count under standard methods using Wright's staining technique (Benjamin, 1995).

3. Histopathological and histochemical observations:

The healing tissue biopsies for histopathological and histochemical examinations were collected on 0 and 3^{rd},7^{th},14^{th},21^{st} and 28^{th} day postoperatively for studying granulation tissue formation, collagen and elastin synthesis. The biopsies were taken from the cutaneous wounds of each group under local infiltration anaesthesia **(2% lignocaine hydrochloride solution)*** without disturbing the wound site with the help of scalpal blade no. 11. These biopsies were fixed in 10% buffered formalin. The segments of the wound tissues submitted for histological examination were trimmed perpendicular to the wound site axis. Paraffin embedded tissue blocks were prepared from each animal and

sections were cut at 5μ thickness. These sections were stained with haematoxylene and eosine stain using standard techniques for healing studies histopathologically (Bancroft and Stevens, 1996).

Special staining techniques like Vangeison's for collagen fibres, Verhoeff's/ Weigert's Resorcin Fuchsin for elastic fibres were used for histochemical studies (Bancroft and Stevens, 1996). Culture sensitivity test (CST) of the wounds was carried out at 7^{th} , 14^{th} , 21^{st} DPT to rule out any infection.

4. Statistical analysis:

Statistical analysis of the data was carried out using analysis of variance method (ANOVA) and comparison between the treatments and days was done at 5% level of significance.

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RESULTS

CHAPTER-4

RESULTS:

The observations of this study are presented as follows:

4.1. Clinical observations

4.2. Haematological observations

4.3. Gross, Histopathological and Histochemical observations

4.1 Clinical observations:

Temperature (Table-1 and Fig.1):

The rectal temperature remained with in the normal range and almost constant throughout the period of observations in all the animals of group I, II and III.

Respiration (Table-2 and Fig.2):

In all the animals of group I, II and III, the respiration rate showed a significant difference ($P<0.05$) in between the groups as well as between the days. The animals of group- I revealed a decrease in the respiration rate on 3rd DPT (day post treatment) whereas a slight increase was observed on 7th DPT and thereafter the respiration remained decreased up to 28 days when compared to the base value(day0).

Table: 1 Effects of various therapeutic regimens on rectal temperature (^{0}F) in cutaneous wound healing in dogs (Mean ± S.E).

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
I (n=3)	102.10 ±0.05	102.06 ±0.06	102.03 ±0.144	102.03 ±0.144	102.16 ±0.115	102.06 ±0.173
II (n=3)	101.40 ±0.646	102.00 ±0.00	102.33 ±0.173	101.93 ±0.173	101.93 ±0.36	102.06 ±0.06
III(n=3) (Control)	101.83 ±0.323	102.03 ±0.479	101.86 ±0.352	101.46 ±0.26	101.63 ±0.184	101.66 ±0.23

* = Day on which the treatment was instituted.
n = number of animals.

Fig.1-Variation in rectal temperature in different groups of animals

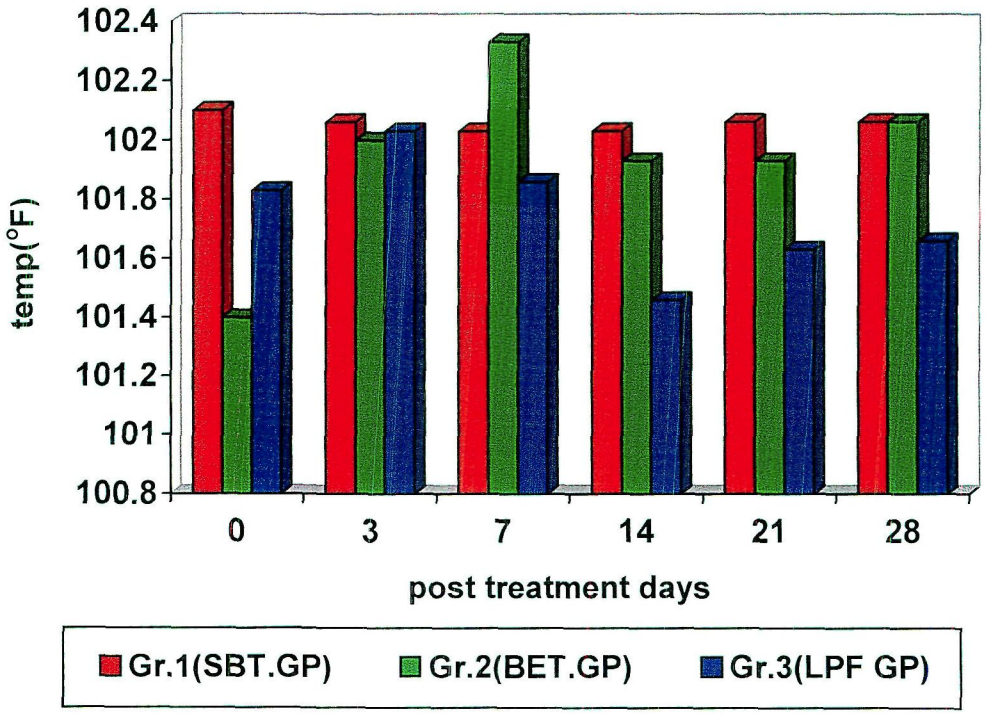
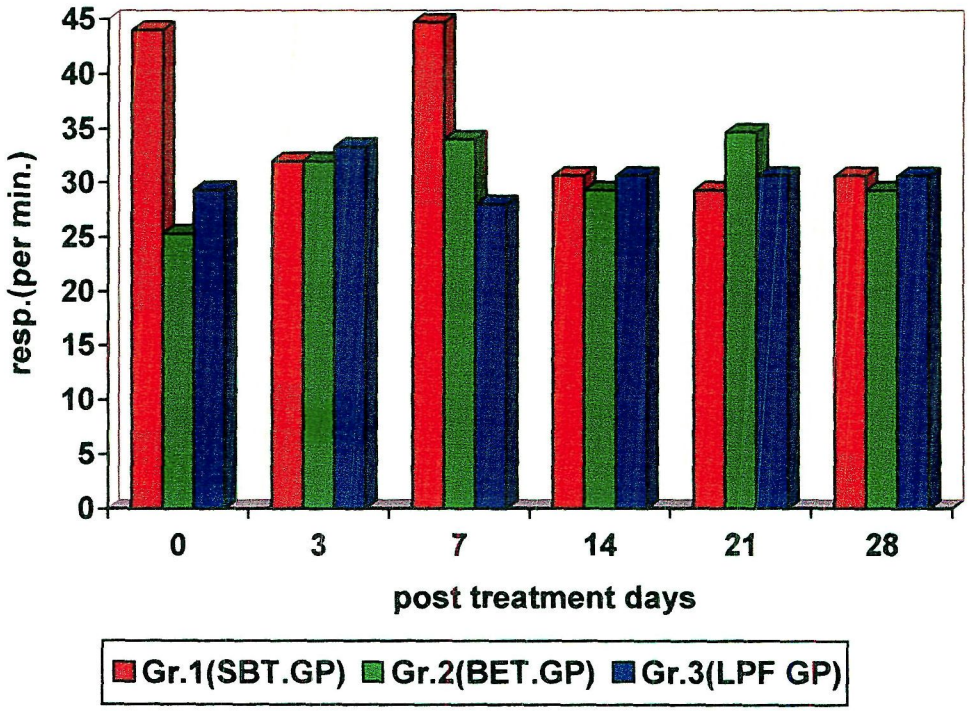


Table: 2 Effects of various therapeutic regimens on respiration rate (per min.) in cutaneous wound healing in dogs (Mean ± S.E.)

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
# I(n=3)	44 ±8.31	32.00 ±3.99	44.66^{A} ±12.64	30.66^{A} ±1.32	29.33 ±1.32	30.66 ±1.32
II (n=3)	25.33 ±1.32	32.00 ±4.61	34.00^{A} ±1.99	29.33^{A} ±1.32	34.66 ±4.80	29.33 ±1.32
III(n=3) (Control)	29.33 ±2.66	33.33 ±3.52	28.00^{A} ±0.00	30.66^{A} ±2.66	30.66 ±1.32	30.66 ±1.32

* = Day on which the treatment was instituted.
n = number of animals.
A indicates P<0.05 when compared with 0 day values.
# indicates P<0.05 when compared with the values of group-II and group-III.

Fig.2- Variation in respiration rate in different groups of animals



In group-II animals, there was an increase in the respiration rate up to 28 DPT when compared with 0 day value. In the animals of control group, the respiration rate was increased on 3rd DPT, but decreased on 7^{th} DPT and thereafter a uniform increase was seen up to 28 days when compared to 0 day.

Heart rate (Table-3 and Fig.3):

The heart rate remained with in the normal range throughout the period of observations in all the animals of group-I, II and III.

Degree of inflammation (Table-4):

The signs of acute inflammatory reactions i.e. swelling, erythema, warmness varied from nil(-) to mild(+) in seabuckthorn and betadine treated groups as compared to control group where the signs were of mild (+) to moderate(++) intensity. The signs of inflammation in the animals of group-I and II subsided by day 3^{rd} post treatment whereas by 7^{th} DPT in the control group.

Appearance of wound exudation (Table-5):

The wound exudation was noticed on 3^{rd} DPT in all the three groups with mild (+) to moderate (++) intensity. The wound exudation almost subsided on 7^{th} DPT in the animals of group-I and group-II as compared to control group where it remained up to 7^{th} DPT with mild (+) to moderate(++) intensity.

Extent of epithelialization (Table-6):

The granulation tissue formation was noticed from the base of the wound on 3^{rd} DPT in seabuckthorn and betadine treated animals as compared to control group where the granulation tissue appeared on 7^{th} DPT. The complete epithelialization and scar formation was observed between 14^{th} and 18^{th} DPT in

Table:3 Effects of various therapeutic regimens on heart rate(per minu in cutaneous wound healing in dogs (Mean±S.E.)

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
I(n=3)	83.33 ±5.69	88.00 ±4.61	86.00 ±7.02	79.33 ±4.05	90.66 ±14.83	84.00 ±3.99
II (n=3)	92.00 ±4.61	100.66 ±6.35	93.33 ±6.66	78.00 ±4.16	98.66 ±11.62	72.00 ±1.15
III(n=3) (Control)	76.00 ±3.99	83.33 ±3.33	85.33 ±5.32	10I.33 ±1.32	76.66 ±2.40	84.00 ±3.99

* = Day on which the treatment was instituted.
n = number of animals.

Fig.3-variation in heart rate in different groups of animals.

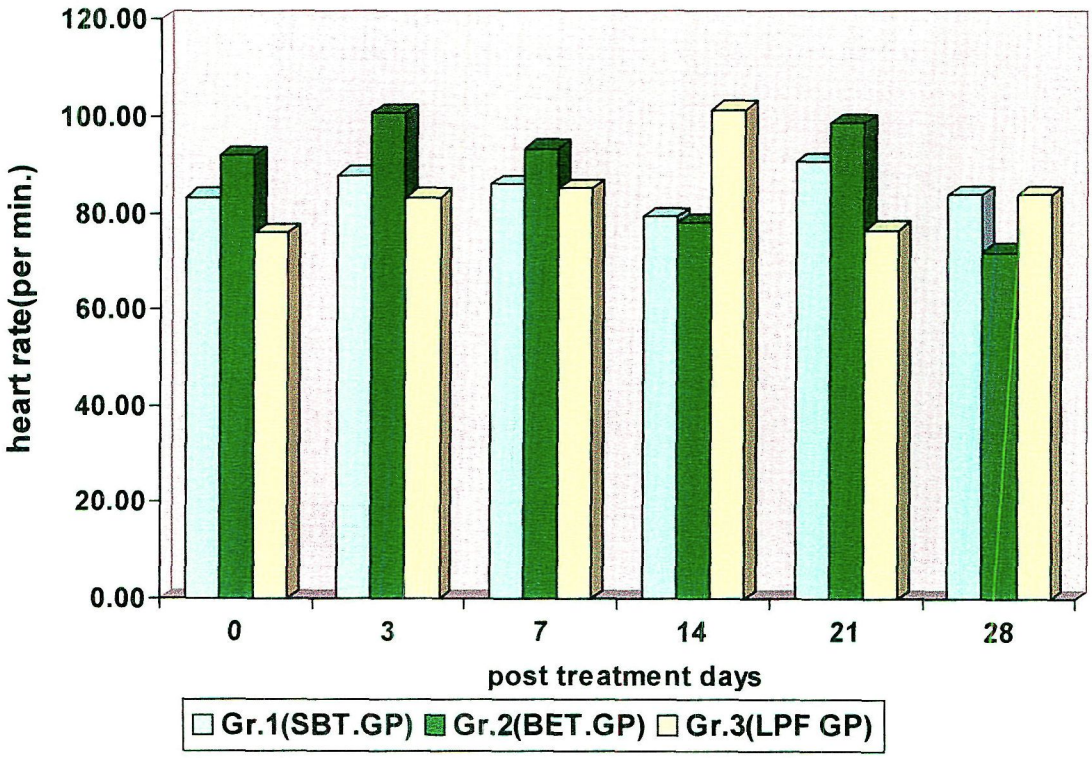


Table-4 Comparative effects of various therapeutic regimens on degree of inflammation in cutaneous wound healing in dogs.

Group	Animal number	0* day (Base value)	Post treatment days				
			3	7	14	21	28
I	1	-	+	-	-	-	-
	2	-	+	-	-	-	-
	3	-	+	-	-	-	-
II	1	-	+	-	-	-	-
	2	-	+	-	-	-	-
	3	-	+	-	-	-	-
III Control	1	-	+	++	-	-	-
	2	-	++	+	-	-	-
	3	-	+	+	-	-	-

*** = day on which the treatment was instituted.**

- = no inflammation

+ = mild inflammation

++ = moderate inflammation

+++= severe inflammation

Table-5 Comparative effects of various therapeutic regimens on appearance of wound exudation in cutaneous wound healing in dogs.

Group	Animal number	0* day (Base value)	Post treatment days				
			3	7	14	21	28
I	1	-	-	-	-	-	-
	2	-	+	-	-	-	-
	3	-	-	-	-	-	-
II	1	-	+	+	-	-	-
	2	-	+	-	-	-	-
	3	-	+	-	-	-	-
III Control	1	-	++	++	-	-	-
	2	-	++	++	-	-	-
	3	-	+	+	-	-	-

* = day on which the treatment was instituted.

\- = no exudation

\+ = mild exudation

++ = moderate exudation

+++= severe exudation

Table-6 Comparative effects of various therapeutic regimens on extent of epithelialization in cutaneous wound healing in dogs.

Group	Animal number	0* day (Base value)	Post treatment days				
			3	7	14	21	28
I	1	-	+	++	+++	+++	+++
	2	-	-	++	+++	+++	+++
	3	-	+	++	+++	+++	+++
II	1	-	+	+++	+++	+++	+++
	2	-	-	++	+++	+++	+++
	3	-	+	+	+++	+++	+++
III Control	1	-	+	+	++	+++	+++
	2	-	-	++	++	+++	+++
	3	-	-	+	++	+++	+++

*** = day on which the treatment was instituted.**

- = no epithelialization

+ = mild epithelialization

++ = moderate epithelialization

+++ = extensive epithelialization

the animals of group-I and II as compared to control group where the complete scar formation was noticed between 21st and 24th DPT. The degree of epithelialization was extensive (+++) in the animals of group-I and group-II as compared to control group where extent of epithelialization was moderate (++).

Percent wound contraction (Table-7 and Fig.4):

Seabuckthorn and betadine (Povidone iodine) treated wounds showed uniform contraction up to 36% and 32% respectively as compared to control group which showed only 17% wound contraction on 7th DPT. The percent wound contraction was up to 75% and 77% respectively in the animals of group-I and II on 14th DPT as compared to control group where the percent wound contraction was up to 69%. By 21st DPT, complete contraction of all the wounds was noticed in all the animals of group-I, II and III.

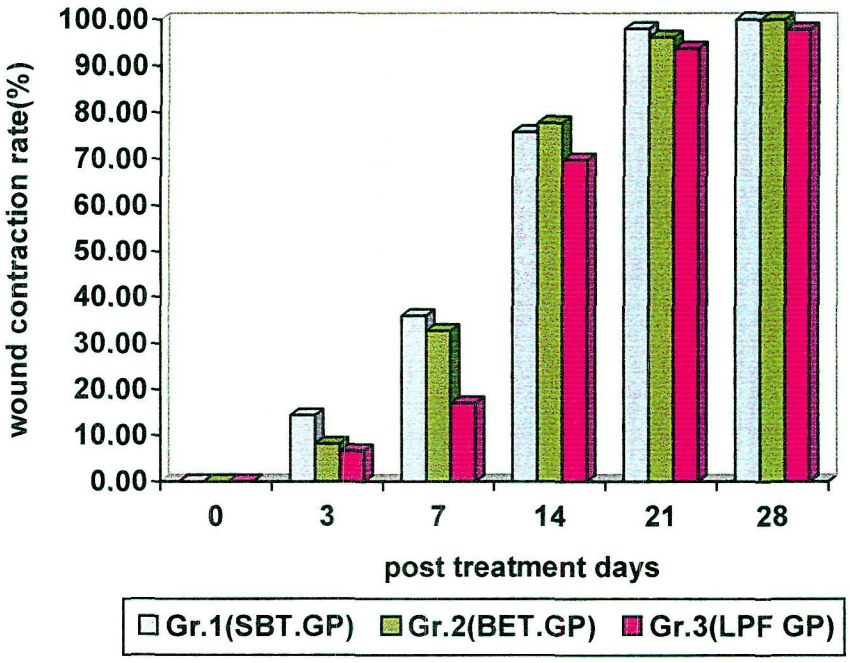
On comparative basis, a significant difference ($P<0.05$) was recorded in percent wound contraction in the animals of group-I and group-II when compared to control group. The wound contraction was more (better) in seabuckthorn and betadine treated wounds than liquid paraffin treated wounds (control). However no significant difference ($P>0.05$) was noticed in percent wound contraction between seabuckthorn and betadine treated animals. A significant difference ($P<0.05$) was observed with respect to days with maximum contraction occurring on 28th DPT in the animals of all the three groups when compared to base value.

Table: 7 Effects of various therapeutic regimens on percent wound contraction rate (%) in cutaneous wound healing in dogs (Mean±S.E.

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
# I(n=3)	0.00 ±0.00	14.33^{A} ±11.14	35.98^{A} ±6.03	75.83^{AX} ±4.27	97.92^{A} ±0.29	100.00^{A} ±0.00
#II (n=3)	0.00 ±0.00	8.26^{A} ±8.26	32.73^{A} ±9.52	77.73^{AX} ±8.86	96.13^{A} ±2.62	100.00^{A} ±0.00
III(n=3) (Control)	0.00 ±0.00	6.66^{A} ±6.63	17.01^{A} ±7.45	69.76^{AX} ±1.76	93.72^{A} ±2.88	97.87^{A} ±0.17

* = Day on which the treatment was instituted
# indicates P<0.05 when compared with group-III (control) values.
A indicates P<0.05 when compared with 0 day values.
X indicates P<0.05 when compared with 3^{rd} and 7^{th} day Values.
n = number of animals.

Fig.4- Variation in percent wound contraction in different groups of animals.



4.2 Haematological parameters:

Haemoglobin (Table-8 and Fig.5):

A significant difference ($P<0.05$) was observed in the haemoglobin values in between the treated groups but no significant change ($P>0.05$) was seen in between the days in all the three groups when compared to day 0.

Packed cell volume (Table-9 and Fig.6):

Haematocrit (P.C.V) values were comparable to the day 0(base value) and no significant difference ($P>0.05$) was found in these values between the treatments as well as between the days.

Total erythrocyte count (Table-10 and Fig.7):

A significant difference was noticed ($P<0.05$) in Total erythrocyte count (TEC) values in between the groups at different time intervals but no significant difference ($P>0.05$) was found between the days. Although the TEC values in seabuckthorn treated animals showed increased count up to 28^{th} DPT but this increase was not significant($P>0.05$). In betadine treated animals, there was a gradual decrease in TEC values up to 14^{th} DPT and then increased TEC values were recorded by day 21^{st} and 28^{th} post treatment as compared to the base values but this change was not significant($P>0.05$). In the animals of control group, a non significant($P>0.05$) decrease in TEC values was observed up to day 7^{th} post treatment and then increase was noticed up to day 28^{th} post treatment as compared to the base value.

Table-8 Effects of various therapeutic regimens on Haemoglobin (gm%) in cutaneous wound healing in dogs (Mean±S.E.)

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
#I(n=3)	13.30 ±1.05	13.13 ±1.27	13.33 ±1.09	13.60 ±0.59	12.73 ±0.54	12.56 ±0.82
II (n=3)	11.13 ±0.69	11.93 ±0.46	11.50 ±0.76	12.20 ±0.57	11.80 ±1.05	11.80 ±0.69
III(n=3) (Control)	11.73 ±1.82	11.40 ±0.86	11.73 ±1.74	11.83 ±0.92	12.23 ±0.86	11.90 ±0.96

* = Day on which the treatment was instituted.
# indicates $P<0.05$ when compared with group-II and group-III (control) values
n = number of animals.

Fig.5 Variation in haemoglobin values in different groups of animals.

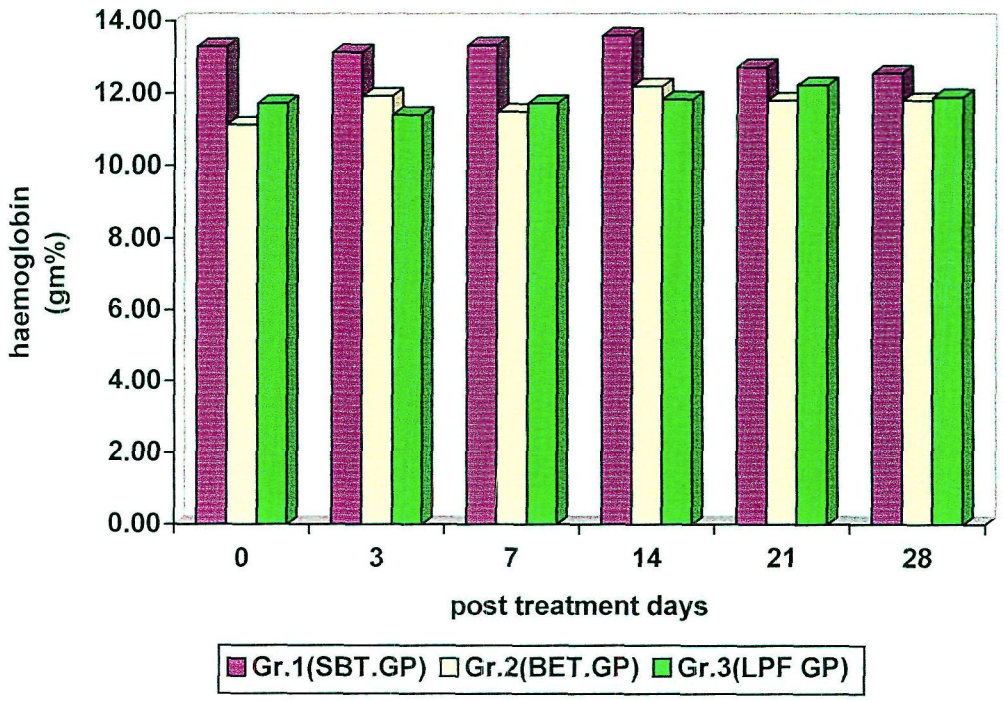


Table-9 Effects of various therapeutic regimens on Packed cell volume (%) in cutaneous wound healing in dogs (Mean±S.E.)

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
I(n=3)	43.00 ±5.85	43.33 ±5.69	44.33 ±6.93	45.00 ±5.19	40.66 ±2.40	41.33 ±0.66
II(n=3)	42.66 ±0.66	40.00 ±1.99	38.66 ±3.33	40.00 ±1.99	42.00 ±1.15	44.00 ±2.30
III(n=3) (Control)	47.33 ±6.35	39.33 ±5.45	39.66 ±4.33	38.00 ±2.30	39.33 ±4.37	38.00 ±3.05

* = Day on which the treatment was instituted.
n = number of animals.

Fig.6-Variation in PCV values (%) in different groups of animals.

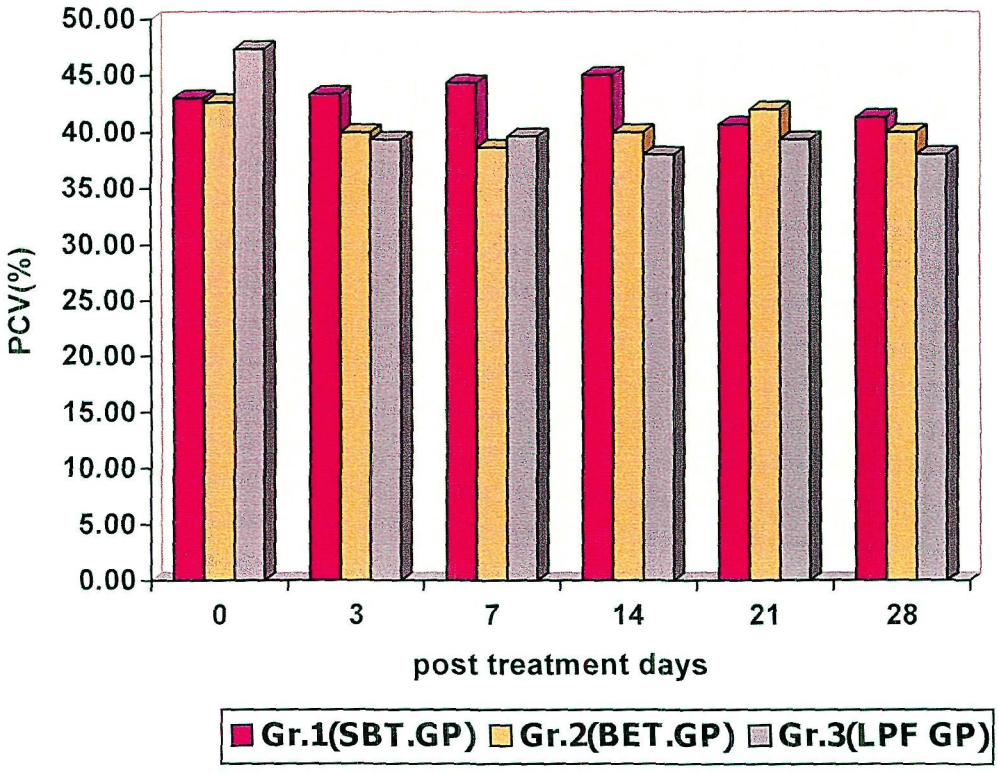
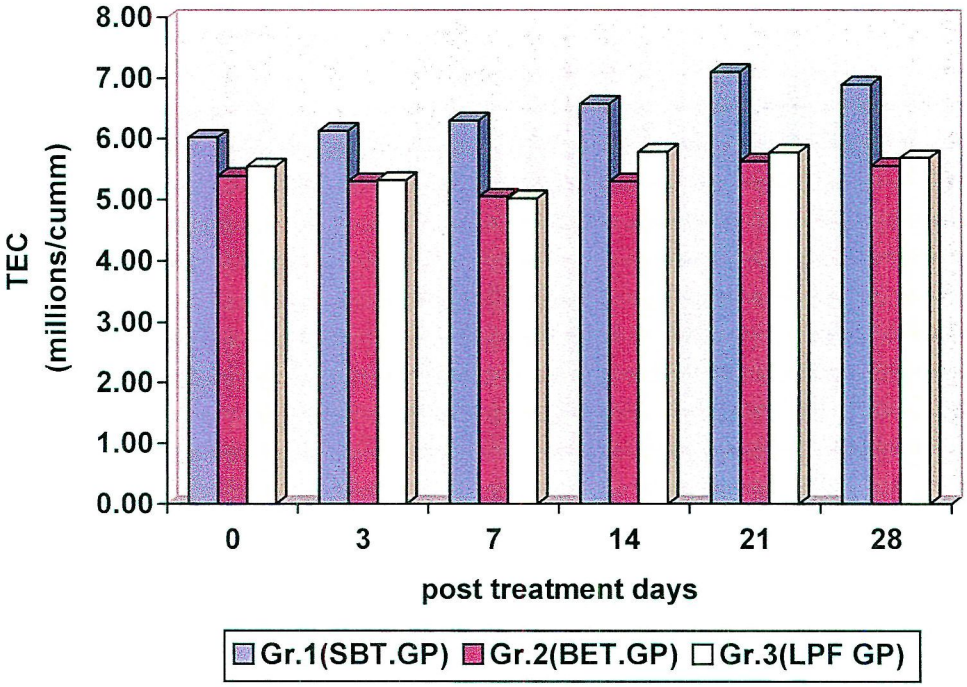


Table-10 Effects of various therapeutic regimens on Total erythrocyte count, in millions/cu.mm) in cutaneous wound healing in dogs. (Mean±S.E.)

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
# I (n=3)	6.02 ±0.42	6.13 ±0.06	6.30 ±0.60	6.58 0.40	7.10 ±0.45	6.90 ±0.37
II (n=3)	5.39 ±1.84	5.30 ±1.50	5.05 ±1.44	5.30 ±1.62	5.63 ±1.70	5.56 ±1.65
III(n=3) (Control)	5.55 ±1.19	5.32 ±0.62	5.02 ±0.48	5.79 ±0.30	5.78 ±0.40	5.69 ±0.15

* = Day on which the treatment was instituted.
n = number of animals.
# indicates P<0.05 when compared with group-II and group-III (control) values.

Fig.7-Variation in TEC values in different groups of animals



Total leucocyte count (Table-11 and Fig.8):

Total leucocyte counts (TLC) were comparable to the base value and no significant difference ($P>0.05$) was found in these values between the treatments as well as between the days. Although a non significant ($P>0.05$) increase in the TLC values were recorded by day 7^{th} post treatment in all the three groups ($P>0.05$).

Differential leucocyte count (Table-12 and Fig.9):

Differential leucocyte count exhibited no significant difference ($P>0.05$) between the treatment groups as well as between the days at different time intervals although all the three groups showed a non significant ($P>0.05$) neutrophilia up to day 3^{rd} post treatment.

4.3 Gross, histopathological and histochemical observations:

Gross observations:

At 3^{rd} DPT (day post treatment):

The signs of acute inflammatory reaction (swelling, pain, erythema and exudation) were noticed in the animals of group –III. The blood tinged exudation noticed in this animal group was of mild (+) to moderate (++) intensity with greyish appearance of its contents (Plate-1). The mild haemorrhagic exudate along with a thick dried scab partially covering the wound surface was observed in the animals of group-II (Plate-2). However in the animals of group-I, the boarders of the wound were somewhat raised and the wound cavity was completely covered by thick semidried scab with no exudation (Plate-3).

Table-11 Effects of various therapeutic regimens on Total leucocyte count, in1000/cu.mm) in cutaneous wound healing in dogs (Mean ± S.E.).

GROUP	0 day* (base value)	Post treatment days				
		3	7	14	21	28
I(n=3)	8.57 ±2.95	7.76 ±2.50	9.39 ±2.13	8.48 ±2.77	8.45 ±2.59	8.06 ±2.33
II (n=3)	7.16 ±1.65	7.03 ±1.48	9.62 ±3.72	8.35 ±2.19	8.11 ±2.59	8.01 ±2.56
III(n=3) (Control)	7.00 ±3.16	6.74 ±2.88	7.16 ±3.04	7.84 ±2.48	7.04 ±2.87	7.43 ±3.03

* = Day on which the treatment was instituted.
n = number of animals.

Fig.8-variation in TLC values in different groups of animals.

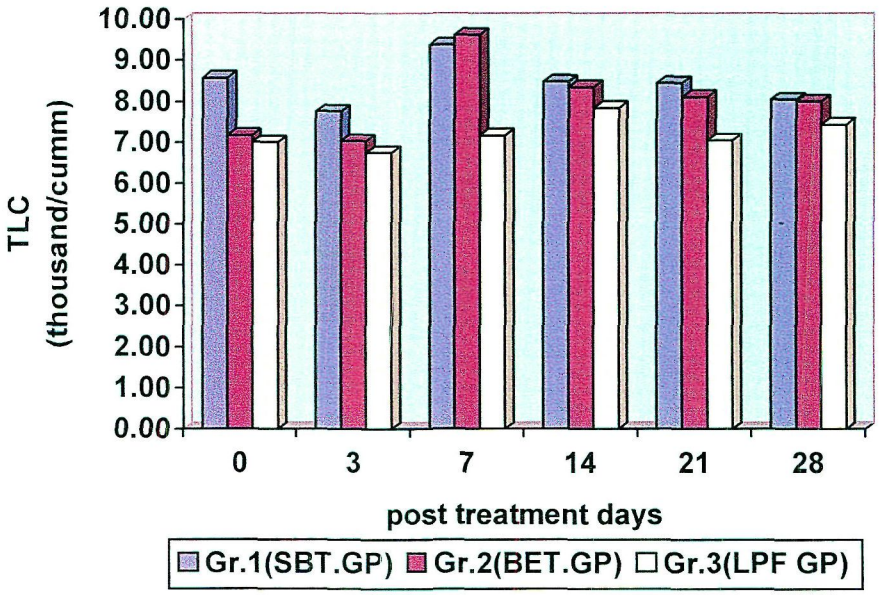
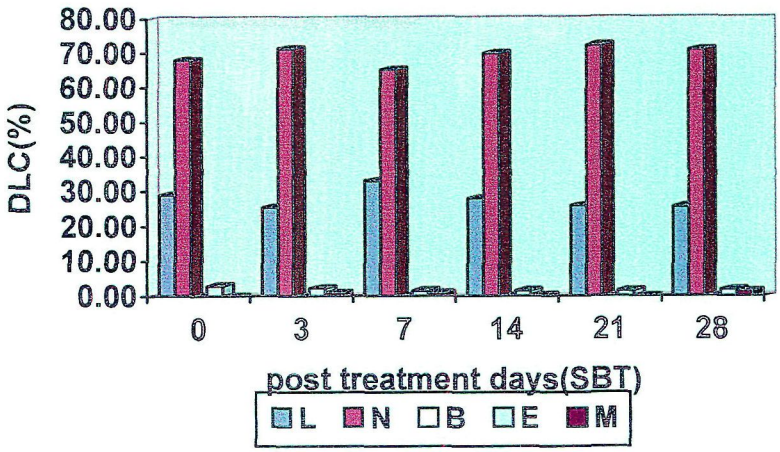


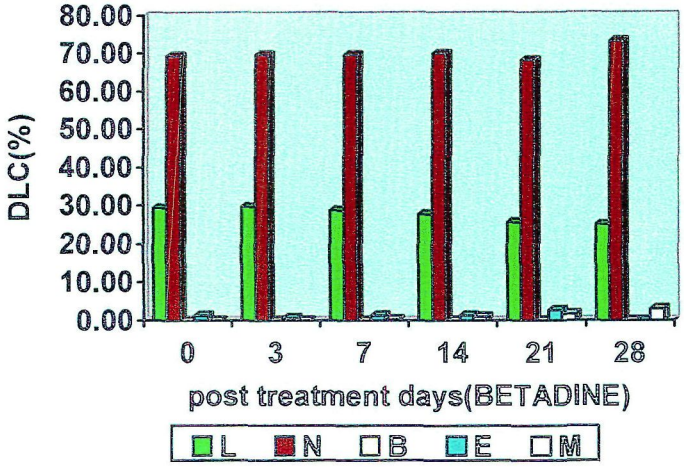
Table-12 Effects of various therapeutic regimens on DLC (differential leucocyte count, %) in cutaneous wound healing in dogs (Mean ± S.E.)

GROUP		0Day* (base value)	Post treatment Days				
			3	7	14	21	28
I (n=3)	L	29.00 ±2.07	25.33 ±1.76	33.00 ±0.81	28.00 ±1.52	26.00 ±3.05	25.66 ±2.33
	N	68.00 ±1.99	71.33 ±3.17	65.33 ±2.90	70.00 ±0.00	72.33 ±1.44	71.00 ±3.78
	B	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
	E	3.00 ±1.52	2.33 ±1.20	1.66 ±1.66	1.66 ±1.66	1.66 ±1.66	2.00 ±1.19
	M	0.00 ±0.00	1.00 ±0.99	1.00 ±0.99	0.33 ±0.32	0.00 ±0.00	1.33 ±1.32
II (n=3)	L	29.33 ±4.09	29.66 ±0.32	28.66 ±2.33	27.66 ±1.44	25.66 ±3.47	25.00 ±0.00
	N	69.33 ±4.09	69.66 ±0.87	69.66 ±1.44	70.00 ±2.88	68.33 ±0.87)	73.33 ±1.66
	B	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00)	0.00 ±0.00
	E	1.33 ±1.32	0.66 ±0.66	1.33 ±1.32	1.33 ±1.32	2.66 ±1.44)	0.00 ±0.00
	M	0.00 ±0.00	0.00 ±0.00	0.33 ±0.32	1.00 ±1.00	1.66 ±0.87)	3.00 ±1.52
III (n=3) control	L	33.33 ±1.76	25.00 ±2.88	28.66 ±2.33	28.33 ±0.87	29.00 ±2.07	29.33 ±0.66
	N	64.66 ±2.66	71.66 ±1.66	69.00 ±0.99	69.33 ±2.33	67.66 ±1.44	66.66 ±4.40
	B	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
	E	3.00 ±0.57	3.33 ±1.66	2.33 ±1.44	1.00 ±0.99	0.00 ±0.00	0.00 ±0.00
	M	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.66 ±0.66	0.66 ±0.66

L – lymphocyte, N – Neutrophil, B – Basophil, E – Eosinophil, M – Monocyte

FIG.9-variation in DLC values in different groups of animals





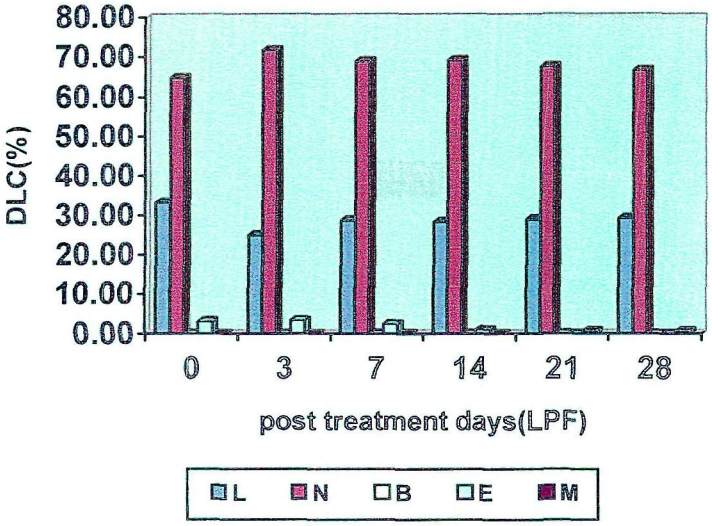


Plate-1: Photograph from LPF group on 3rd DPT showing wound with blood tinged exudate and greyish appearance of its contents.

Plate-2: Photograph from BET group on 3rd DPT showing wound with plenty of haemorrhagic exudate along with partial covering by a thick dried scab.

Plate-3: Photograph from SBT group on 3rd DPT showing wound with somewhat raised boarders and was completely covered by thick semidried scab.

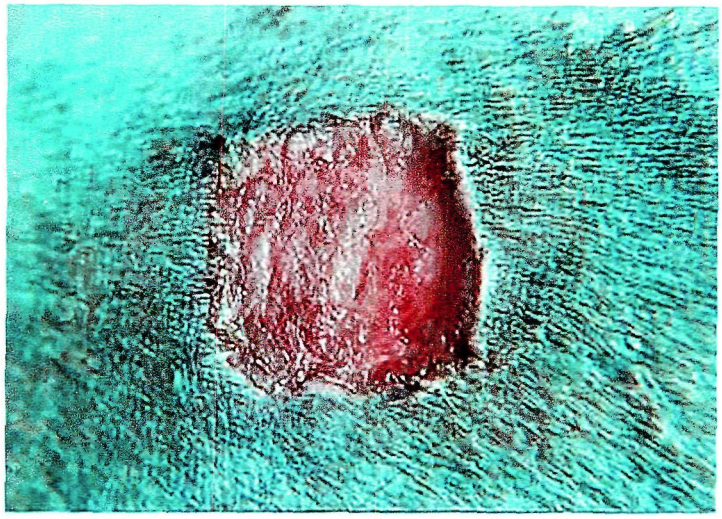


Plate 1

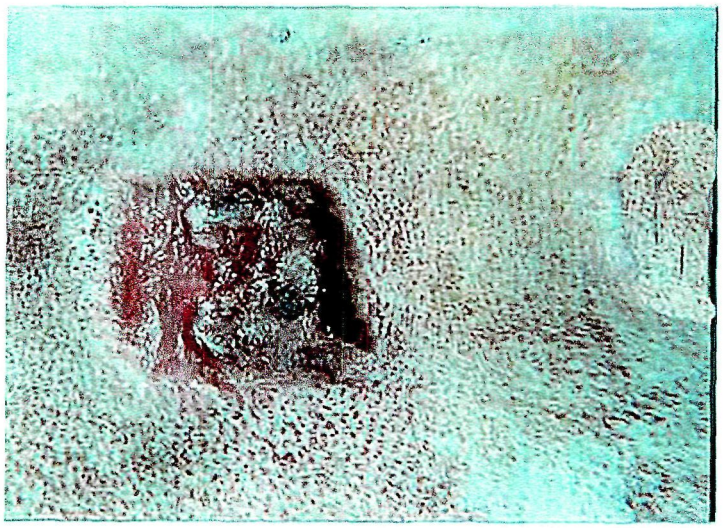


Plate 2

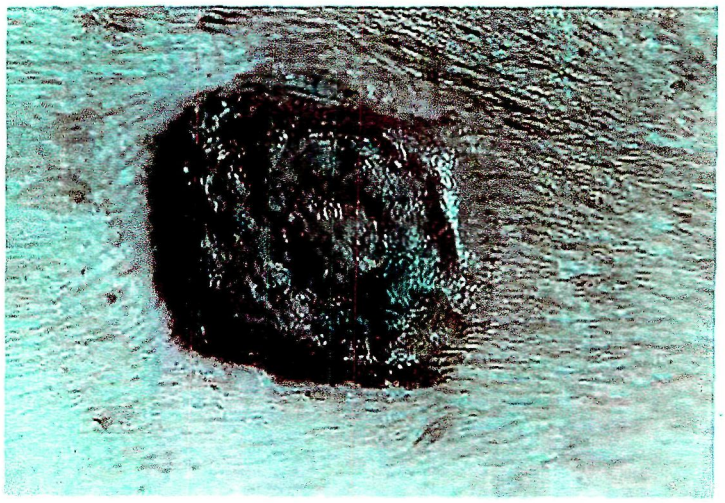


Plate 3

At 7th DPT:

The signs of inflammation in the animals of group-I and II subsided completely by day 3rd post treatment as compared to control group animals where the signs of inflammation(mild exudation and hyperaemia) were still present on 7th DPT along with increased granular appearance of the healing tissue(Plate-4). The wound cavity was covered completely by a thin moist scab in the animals of group-II (Plate-5) and by a thick dried scab in the animals of group-I (Plate-6).

At 14th DPT:

In the animals of group-III, the wound showed marked hyperaemia and partial epithelialization of the wound surface along with narrowing of the wound gap (Plate-7). In the animals of group-II at this stage beginning of scar formation along with contraction of the wound was a distinct feature (Plate-8) along with covering of the wound gap with a thick deep brown scab.

In the animals of group-I, the contraction of wound along with scar formation was noticed. The central portion of the wound revealed brown scab covering the homogeneous, healthy, pink and firm granulation tissue (Plate-9).

At 21st and 28th DPT:

The wound gap was filled up to the extent of 90% in all the animals of group-I, II and III. The wound in the animals of group-III showed scar formation along with marked epithelialization of the wound surface. The central portion of the wound was covered by a thick immature scab (Plate-10). The shedding of this dried crust occurred between 26th-28th DPT.

Plate-4: Photograph from LPF group on 7th DPT showing wound with mild exudation, hyperaemia and increased granular appearance of the healing tissue.

Plate-5: Photograph from BET group on 7th DPT showing complete covering of the wound by a thin moist scab.

Plate-6: Photograph from SBT group on 7th DPT showing wound cavity completely covered by thick dried scab.

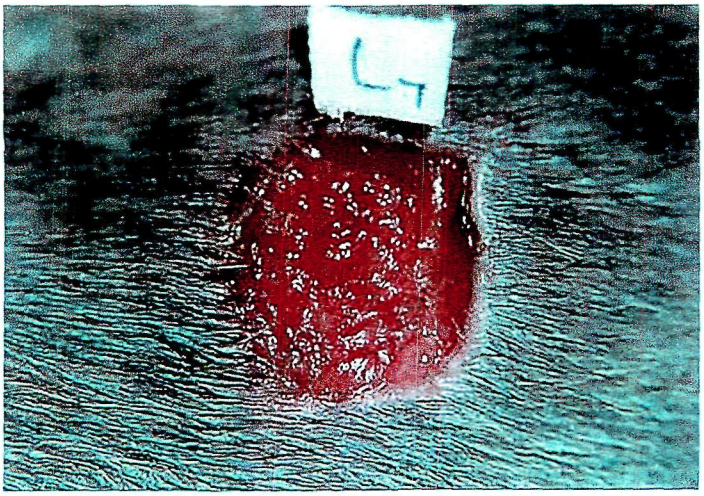


Plate 4

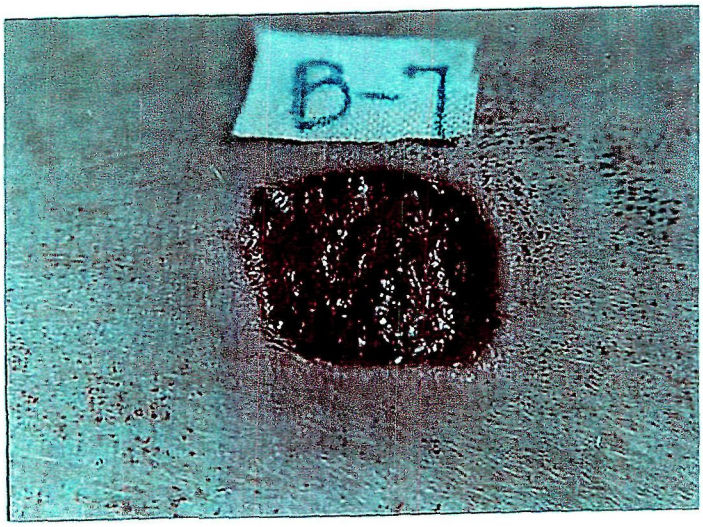


Plate 5

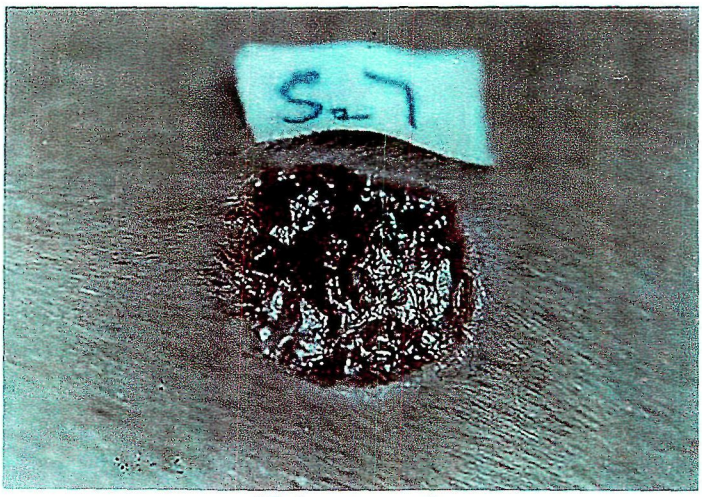


Plate 6

Plate-7: Photograph from LPF group on 14th DPT showing wound with marked hyperaemia and partial epithelialization of the wound surface along with narrowing of the wound gap.

Plate-8: Photograph from BET group on 14th DPT showing beginning of scar formation along with wound contraction and the wound cavity was filled up by a deep brown scab.

Plate-9: Photograph from SBT group on 14th DPT showing beginning of the scar formation and a deep brown scab covering the homogeneous healthy pink and firm granulation tissue along with contraction of wound.

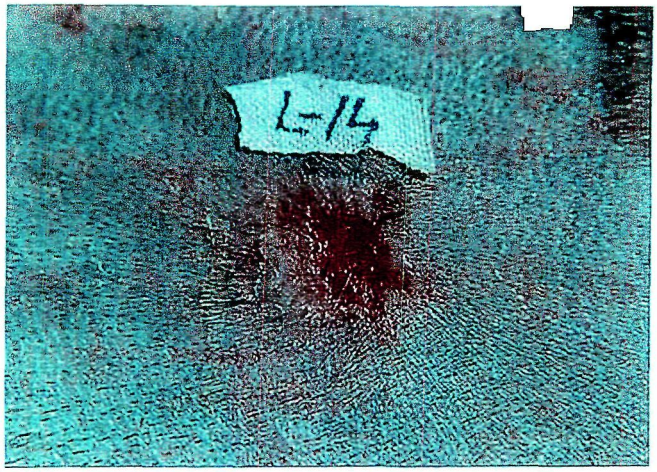


PLATE 7

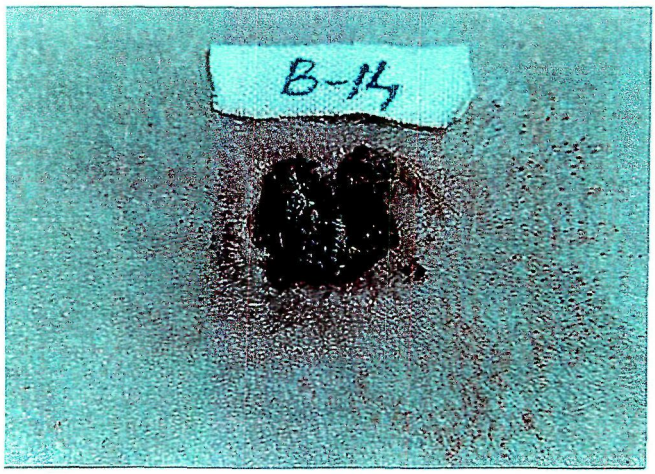


PLATE 8

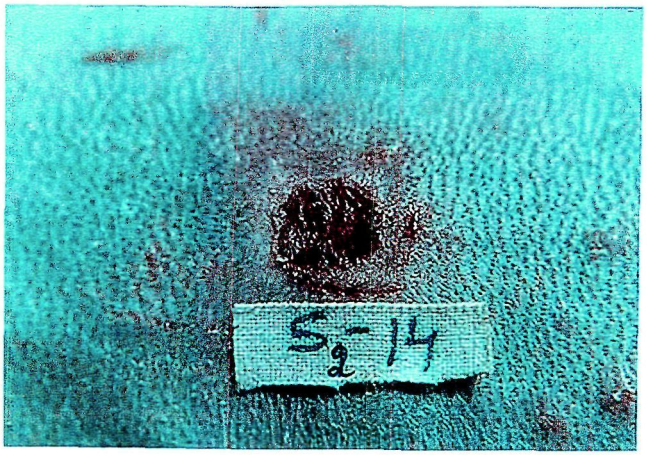


PLATE 9

In group-II animals, there was a distinct scar formation with more or less complete epithelialization of the wound surface (Plate-11). However in the animals of group-I, there was complete formatior of dense scar along with complete epithelialization filling the wound cavity. The shedding of dried crust was almost completed by day 21st post treatment in the animals of group-I as compared to group-II where shedding of the crust was noticed between 23rd-25th DPT (Plate-12). So grossly the control wounds healed slowly when compared with seabuckthorn and betadine treated wounds.

Histopathological and Histochemical observations:

At 3rd DPT:

Liquid paraffin group:

The microscopic changes at 3rd day post treatment were characterized by inflammatory phase with heavy infiltration of leucocytes predominantly with neutrophils. The neutrophillic infiltration was more pronounced on the surface area while it extended with varying severity to the deeper parts as well (Plate-13). Occasional macrophages were also seen. There was plenty of fibrino-purulent exudate which extended even up to deeper parts of wound. The fibrino-cellular clot on the surface of wound also contained large number of RBC's. The blood vessels appeared congested. The area of the wound adjoining the normal epidermis also revealed leucocytic infiltration.

Betadine group:

The superficial areas of the wound appeared necrosed and were heavily infiltrated with neutrophils and RBC's while fibrinopurulent exudation

Plate-10: Photograph from LPF group on 21st DPT showing formation of scar along with marked epithelialization of the wound surface. The central portion of the wound was covered by thick dried scab.

Plate-11: Photograph from BET group on 21st DPT showing scar with more or less complete epithelialization of the wound surface.

Plate-12: Photograph from SBT group on 21st DPT showing complete scar formation along with complete epithelialization and filling of the wound cavity.

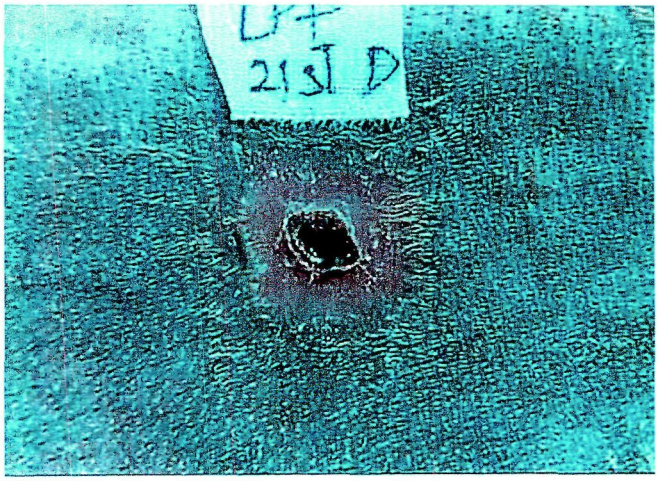


Plate 10

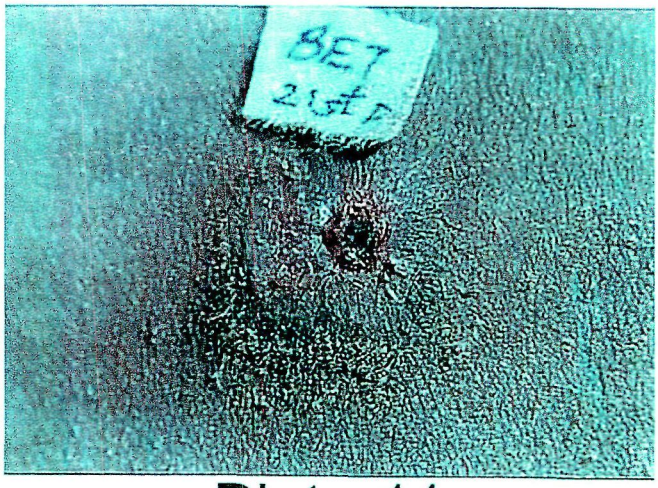


Plate 11

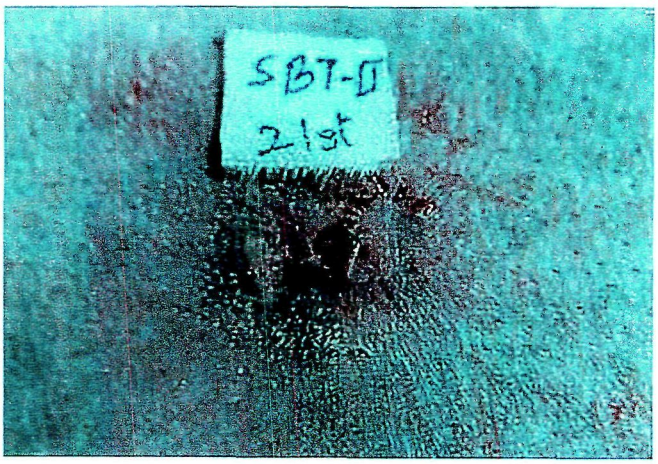


Plate 12

and haemorrhages were comparatively more pronounced in this group (Plate-14). The inflammatory reaction was comparable to the LPF group on 3rd day post treatment. The majority of cells were neutrophils but few macrophages were also observed.

In another animal, however, the inflammatory reaction was restricted to only superficial areas of the wound. Although majority of cells were neutrophils but macrophages were also observed in significant numbers.

Seabuckthorn group:

In this group, there was marked leucocytic infiltration in the superficial areas of the wound. The inflammatory cells included both neutrophils and macrophages. Occasional fibroblasts were also noticed. The exudation was mild when compared to betadine group on 3rd day post treatment (DPT).

Neutrophilic infiltration was mainly confined to the superficial necrotic areas of the wound while the deeper tissues showed mixed infiltration with neutrophils, macrophages and few fibroblasts. Large numbers of capillaries were also seen packed with RBC's immediately below the superficial necrotic area.

At 7th DPT:

Liquid paraffin group:

Inflammatory reaction was more pronounced at 7th DPT. The superficial area of the wound showed marked angiogenesis, infiltration with the leucocytes comprising neutrophils and macrophages (Plate-15). Fibroblasts were evident along with the leucocytes. The deeper parts of the wound showed young granulation tissue with large number of blood vessels opened in this area along

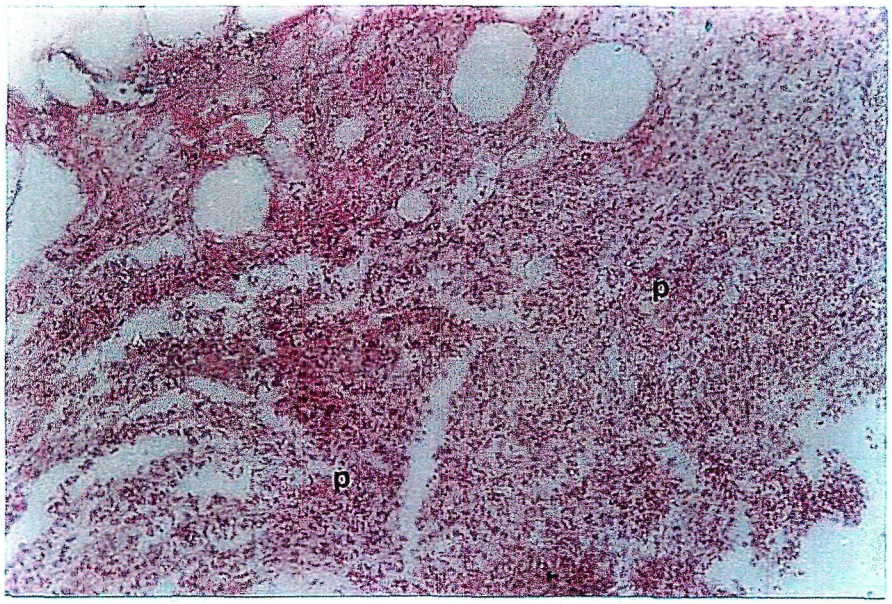


Plate-13: Photomicrograph from LPF group on 3rd DPT showing heavy infiltration with neutrophils (p) in the wounds.

H & E X 66

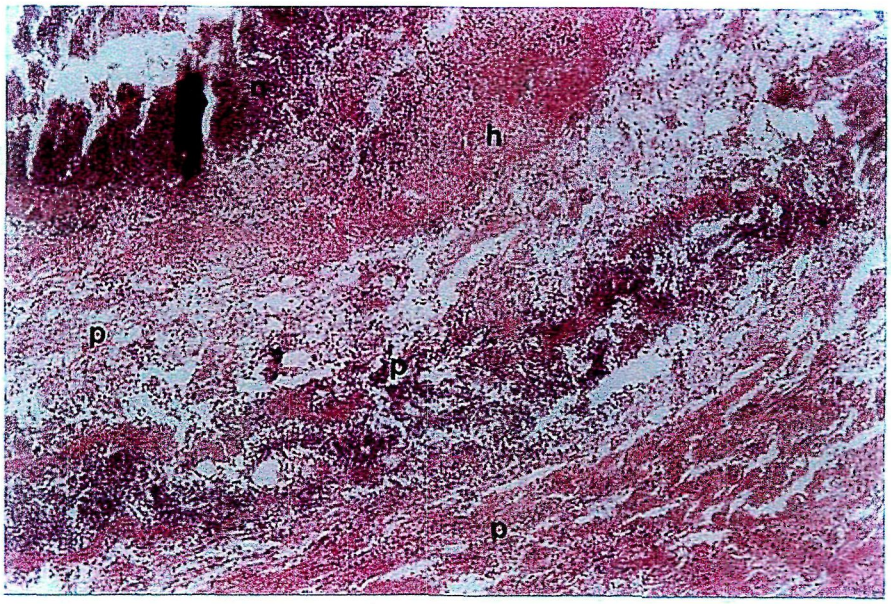


Plate-14: Photomicrograph from BET group on 3rd DPT showing superficial necrotic area infiltrated with neutrophils (n), haemorrhages (h) and fibrinopurulent exudation (p).

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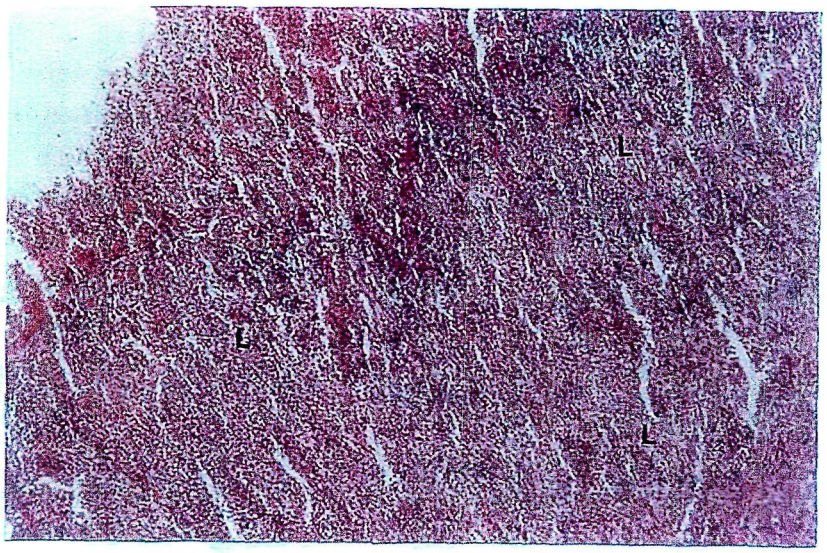

Plate-15: Photomicrograph from LPF group on 7th DPT showing marked infiltration with the leucocytes (L) predominantly the neutrophils and macrophages.

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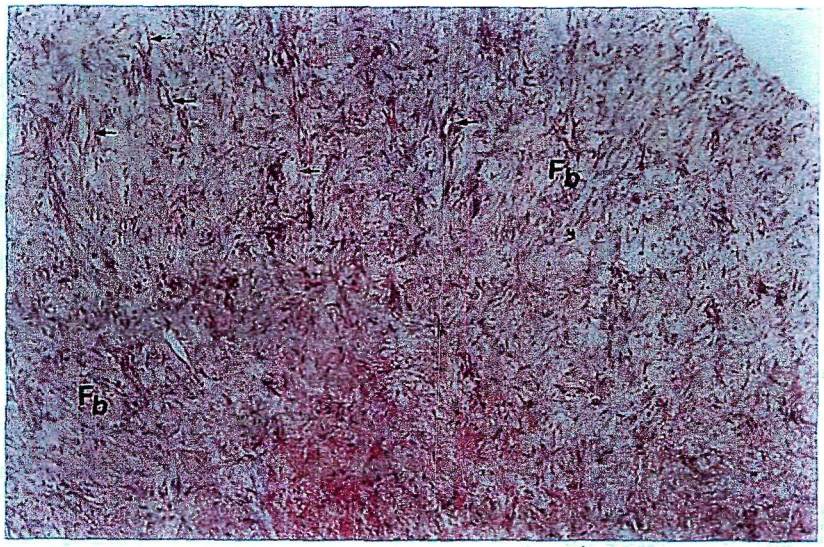


Plate-16: Photomicrograph from LPF group on 7th DPT from the same wound (plate-15) showing granulation tissue containing somewhat disoriented fibroblasts (F_b) and vascular beds (arrows).

H & E X 33

with young fibroblasts which predominantly filled the healing tissue (Plate-16). There were also good numbers of macrophages along with the proliferating fibroblasts particularly in the deeper areas whereas the superficial areas were massively infiltrated with neutrophils followed by a thin layer of proliferating capillary loops. In another animal, neutrophilic infiltration was confined to a thin band on the superficial layer of the wound along with necrotic debris and degenerating cellular elements (Plate-17). The number of capillary beds was much less in the area of collagen fibres in the deeper parts of the wound than what seen immediately below the superficial covering of the wound infiltrated with neutrophils.

Betadine group:

The biopsy from this wound tissue at 7^{th} DPT indicated marked infiltration with leucocytes predominantly with the neutrophils; however occasional macrophages were also noticed in deeper parts of the tissue (Plate-18). Haemorrhages were also seen at some places. Fibroblast proliferation was more as compared to LPF group at this stage but collagen fibres were not laid yet.

The superficial part of the wound comprised of unorganized blood clot and necrotic debris infiltrated with neutrophils while the deeper parts of the wound were found replaced by proliferating fibroblasts and the macrophages predominantly (Plates-19,20).

Angiogenesis was a feature particularly immediately below the necrotic tissue and in the area of inflammation. The epidermis adjoining the

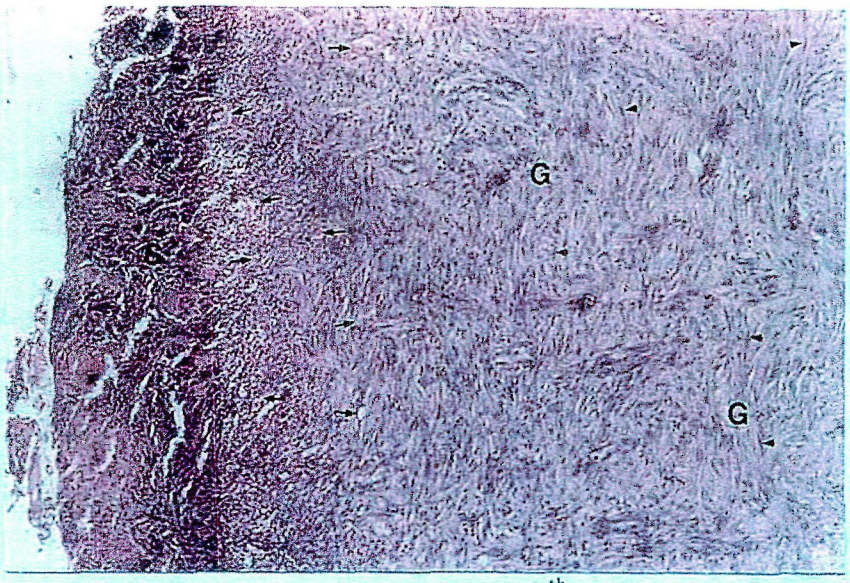


Plate-17: Photomicrograph from LPF group on 7th DPT from other animal showing superficial necrotic area(s) infiltrated with neutrophils followed by a zone of capillary loops(arrows) along with inflammatory cells and granulation tissue(G) chiefly comprising the fibroblasts (arrowheads).

H & E X 33

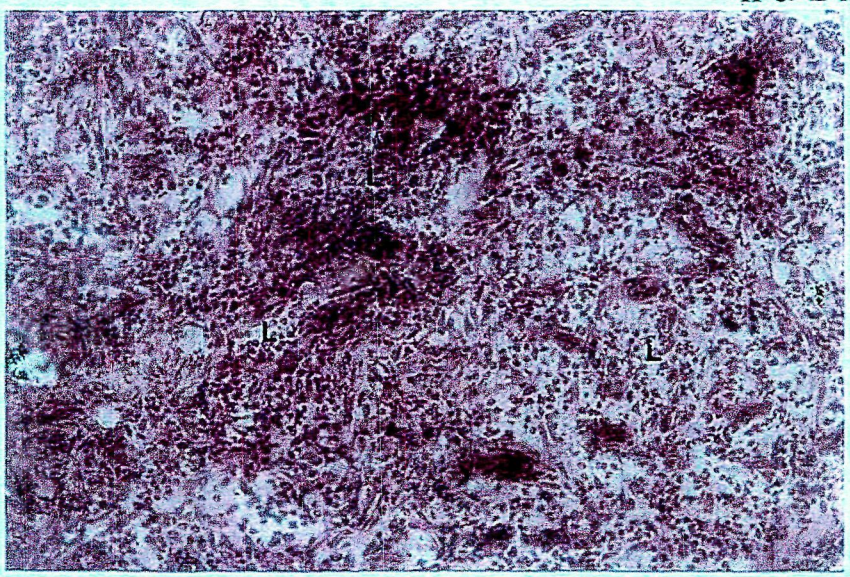


Plate-18: Photomicrograph from BET group on 7th DPT showing marked infiltration with leucocytes (L) predominantly the neutrophils and occasional macrophages.

H & E X 66

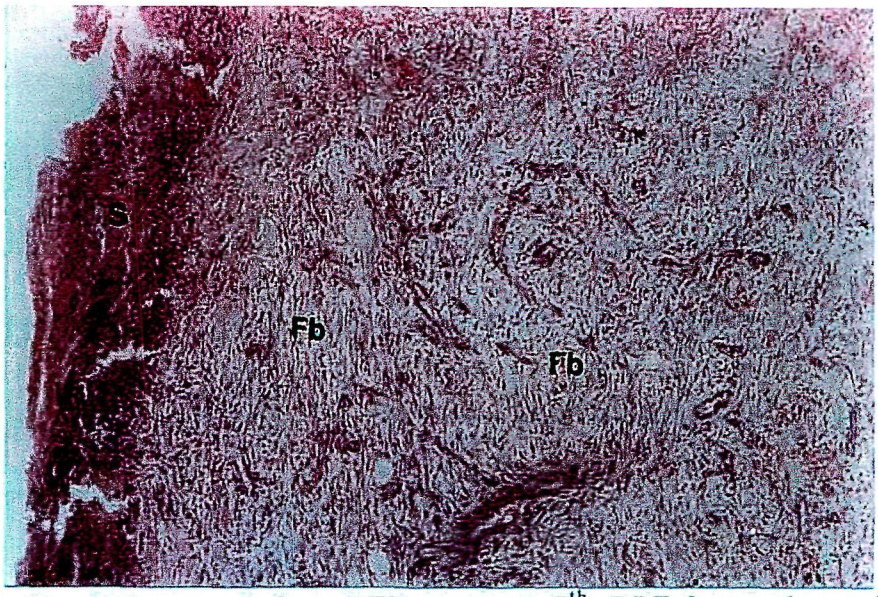


Plate-19: Photomicrograph from BET group on 7th DPT from other animal showing superficial layer of necrotic mass(s) infiltrated with neutrophils followed by granulation tissue comprising proliferating fibroblasts (F_b) and a large number of capillary loops. **H & E X 33**

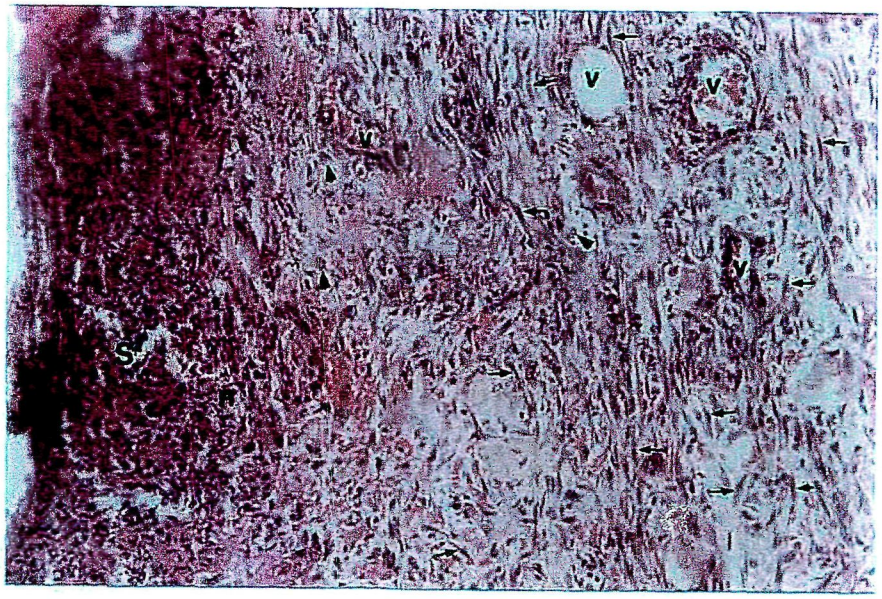


Plate-20: Photomicrograph with higher magnification from the same wound (plate-19)) on 7th DPT showing superficial necrotic layer(s) infiltrated with neutrophils(n) followed by granulation tissue with large number of blood vessels(v), fibroblasts(arrows) at different stages of maturation, neutrophils and mononuclear cells(arrowheads). **H & E X 66**

wound showed hyperplastic changes and the epithelial tissue was found covering the granulation tissue which was relatively less vascular and mature.

Seabuckthorn group:

In this group at 7th DPT, the inflammatory reaction was less pronounced when compared to that in the other two groups. The superficial area of the wound was infiltrated with neutrophils. The angiogenesis was also comparatively more in this group as compared to other two groups.

The granulation tissue comprised mainly fibroblasts but deeper parts were found replaced by mature collagen fibres (Plates-21, 22). There was scattered infiltration with macrophages along with proliferating fibroblasts.

At 14th DPT:

Liquid paraffin group:

Epidermis over the healed tissue appeared flat and hyperplastic and healed tissue was filled up by the proliferating fibroblasts and macrophages. Blood capillaries were also prominent over the area. Occasional neutrophils were also noticed at few places only. Normal adnexal structures of the skin were not seen in the tissue undergoing repair.

In another animal only superficial thin band of necrotic tissue was found infiltrated with neutrophils and a number of blood vessels and capillaries were visible in this area while underneath tissue revealed mainly fibrous tissue associated with infiltration of macrophages (Plates-23, 24). Occasional neutrophils were also noticed along with the fibrous tissue.

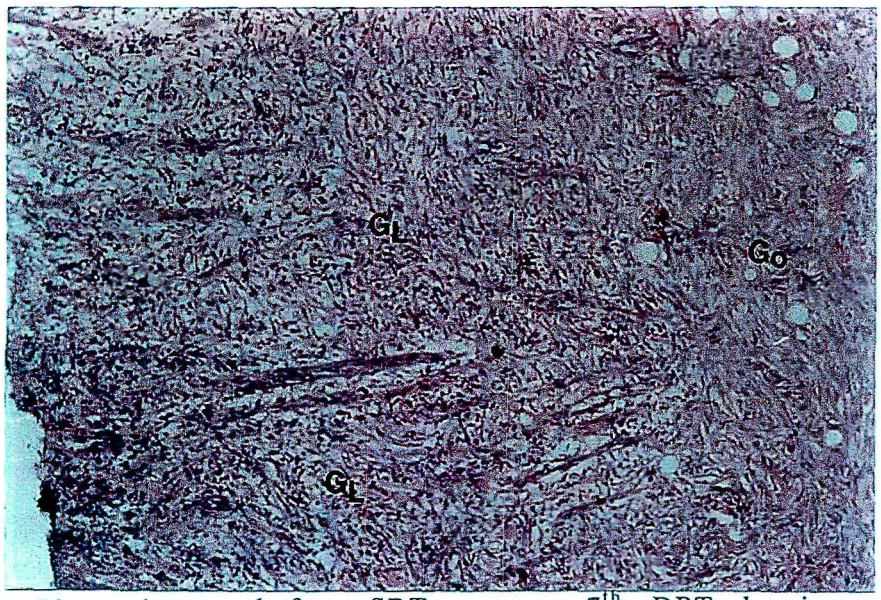


Plate-21: Photomicrograph from SBT group on 7th DPT showing young granulation tissue with large number of leucocytes(G_L) predominantly the neutrophils in between the proliferating fibroblasts while the granulation tissue in the deeper parts appeared organized(G_O) with large number of fibroblasts and a few mature fibres. **H & E X 33**

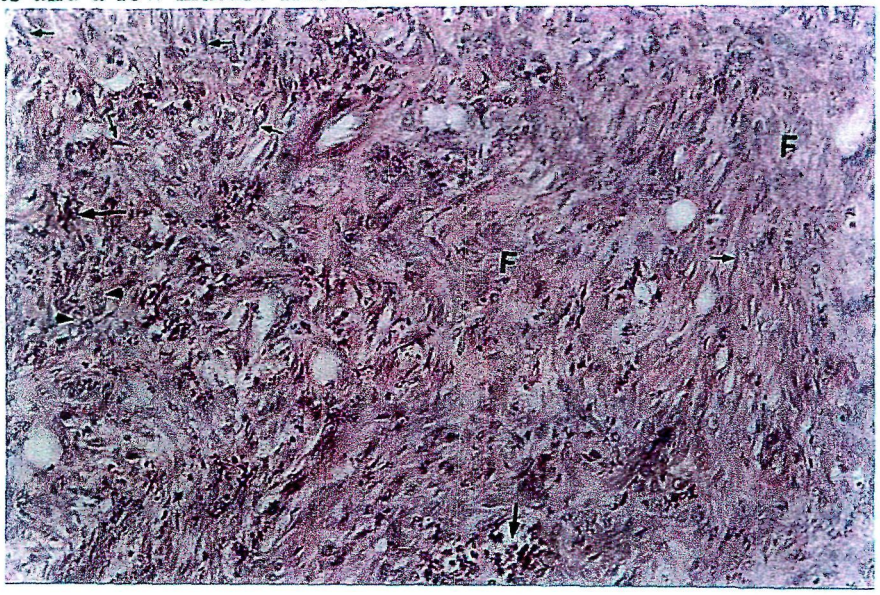


Plate-22: Photomicrograph with higher magnification from the same wound (plate-21) on 7th DPT showing relatively mature fibrous tissue (F) along with the fibroblasts (small arrows), neutrophils (big arrows) and macrophages (arrowheads).

H & E X 66

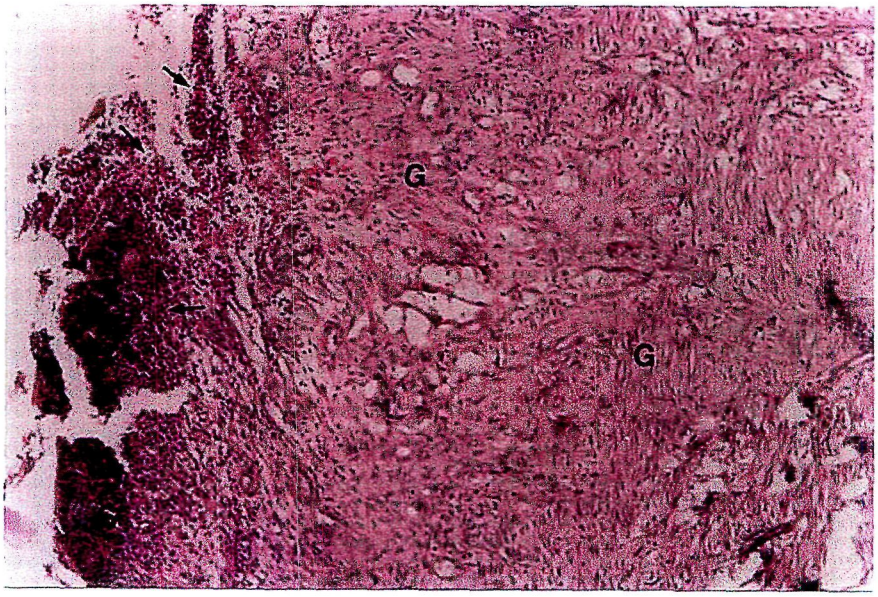


Plate-23: Photomicrograph from LPF group on 14th DPT showing superficial thin band of necrotic tissue(s) infiltrated with neutrophils (arrows) covering the granulation tissue (G) with large number of proliferating blood vessels and fibrous tissue. **H & E X 33**

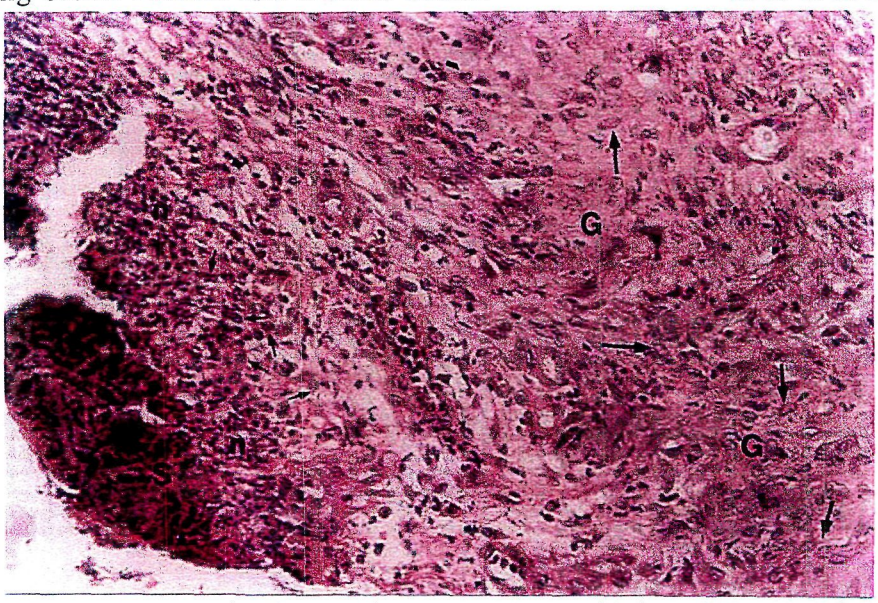


Plate-24: Photomicrograph from the same wound(plate-23) at higher magnification on 14th DPT showing superficial necrotic mass(s) followed by zone of neutrophilic infiltration(n) and macrophages(small arrows) along with large number of capillaries just above the granulation tissue(G) comprising disoriented fibrous tissue(Big arrows). **H & E X 66**

Betadine group:

The response was quite variable in all the animals examined. The inflammatory reaction was more pronounced in one animal with plenty of superficial necrotic tissue heavily infiltrated with neutrophils at 14th DPT (Plate-25). Besides angiogenesis, there was marked infiltration with macrophages extending to the deeper parts of the wound (Plate-25). Epithelium began to flatten out and epidermal hyperplasia was also a distinct feature at this stage.

In the second animal, most of the wound gap was found filled by the collagen fibres particularly in the deeper parts when the sections were stained by vangeison's stain (Plate-26). A thick flat layer of epithelium covered the granulation tissue which also comprised fibroblasts with occasional macrophages besides collagen fibres.

The granulation tissue was much less as compared to LPF group. Inflammatory reaction was relatively more pronounced with large number of neutrophils when compared with LPF group. In another animal, exudative changes containing plenty of RBC's, neutrophils and degenerated cellular elements were noticed while deeper parts of wound also revealed young fibroblasts (Plate-27).

Seabuckthorn group:

The response to the treatment was better in this group. The wound gap was found completely filled by granulation tissue comprising mainly of mature collagen fibres and occasional fibroblasts (Plates-28, 29). The granulation tissue was found covered by a very thin layer of necrotic tissue which was

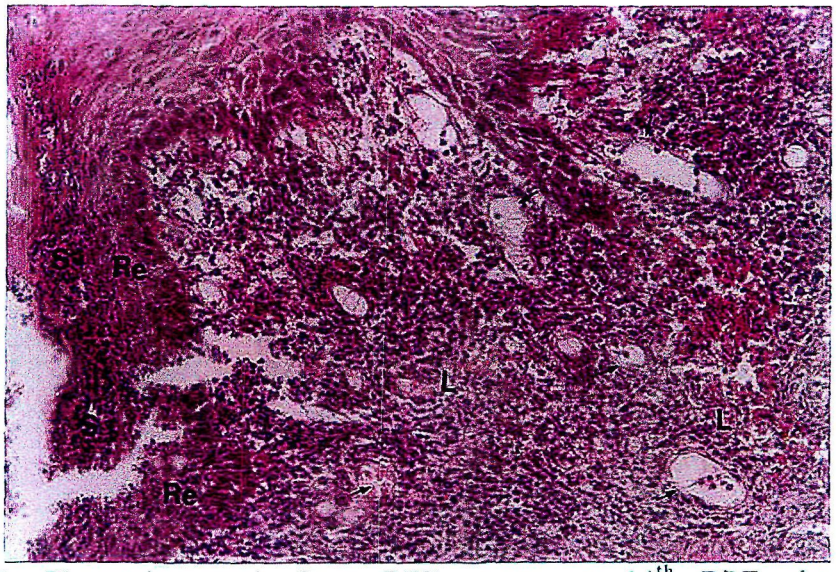


Plate-25: Photomicrograph from BET group on 14th DPT showing epidermal hyperplasia and regenerating epithelium (R_e) underneath superficial layer of necrotic tissue(s). The healing tissue was largely filled by infiltrating leucocytes (L) and proliferating blood vessels (arrows)

H & E X 66

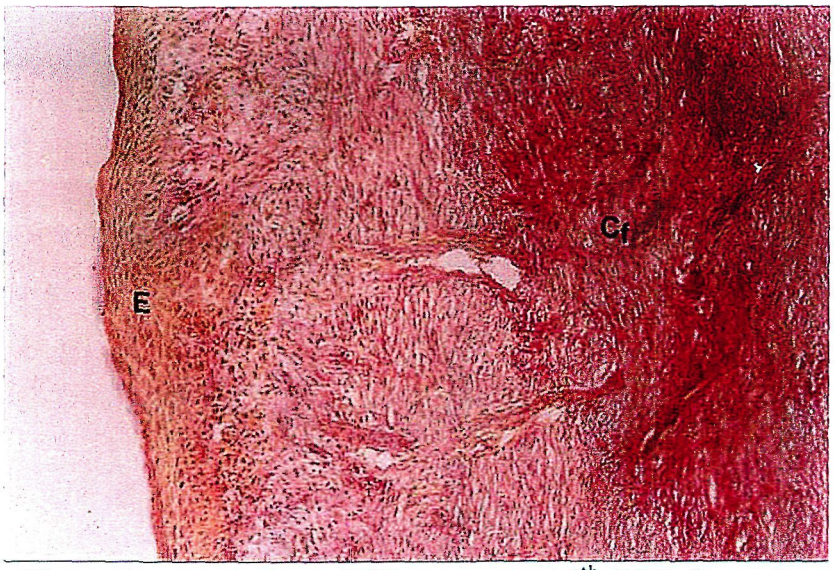


Plate-26: Photomicrograph from BET group on 14th DPT from the other animal showing flat layer of epithelium (E) with absence of rete ridges and replacing of the deeper parts of the wound by collagen fibres (C_F).

Vangeison X 33

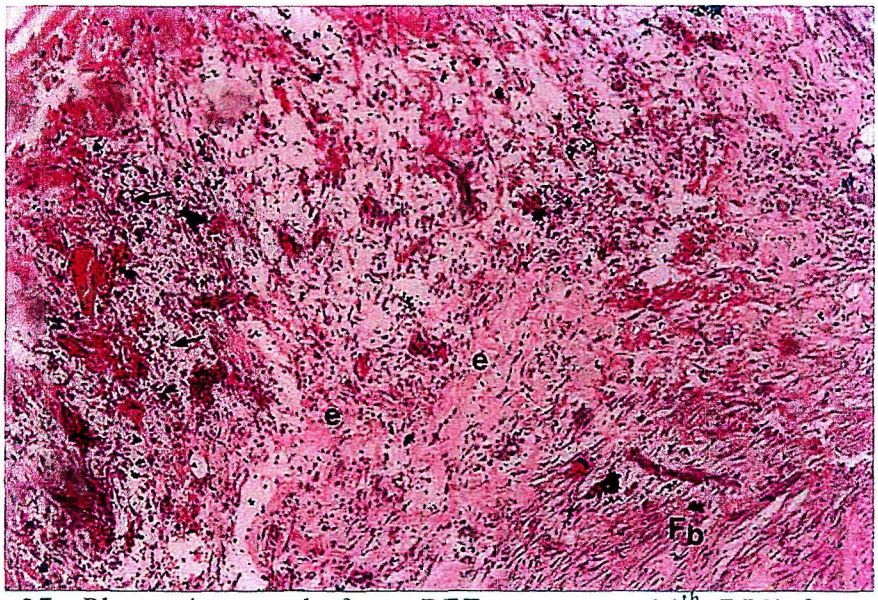


Plate-27: Photomicrograph from BET group on 14th DPT from another animal showing exudate (e), neutrophils (arrows) and fibroblast proliferation (F_b) in relatively deeper parts of the wound.

H & E X 33

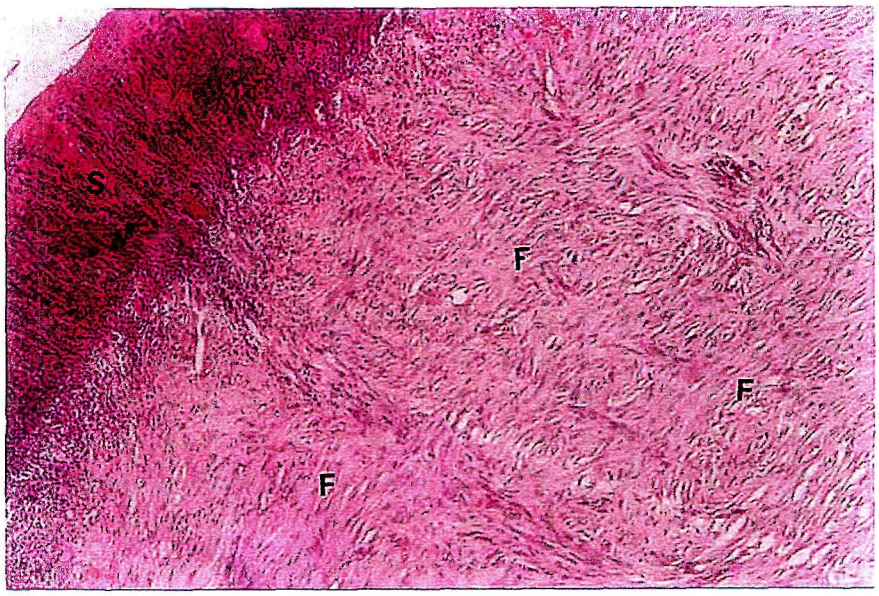


Plate-28: Photomicrograph from SBT group on 14th DPT showing superficial necrotic tissue infiltrated with neutrophils and degenerated cellular elements(s) covering the mature fibrous tissue (F) which appeared more densely packed and organized.

H & E X 33

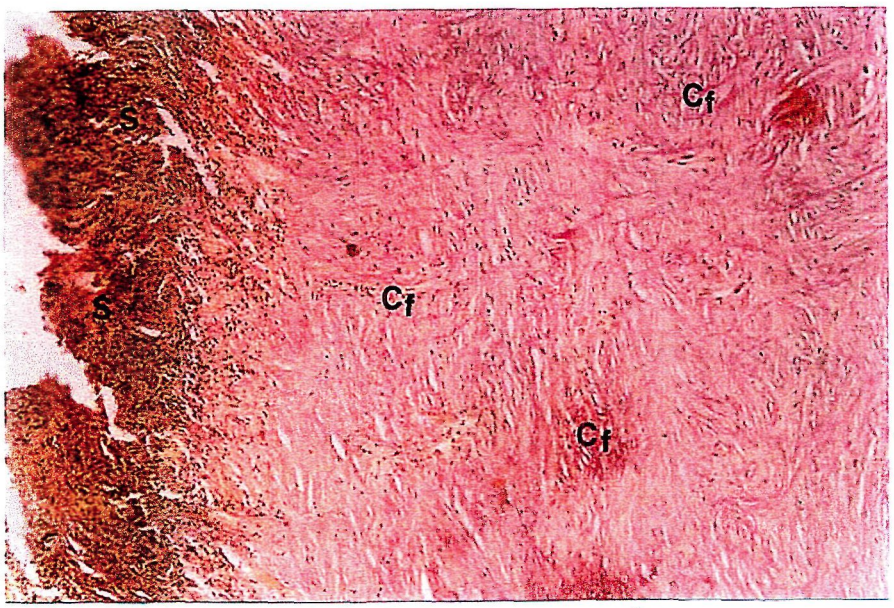


Plate-29: Photomicrograph from SBT group on 14th DPT from the same wound (plate-28) showing a thin layer of necrotic tissue(s) and pink staining collagen fibres (C_F) filling the wound gap.

Vangeison X 33

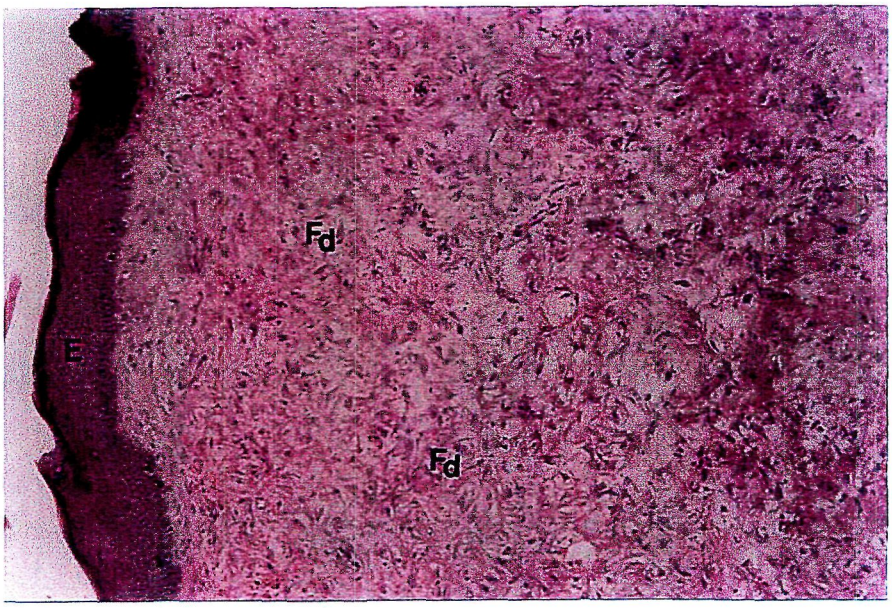


Plate-30: Photomicrograph from LPF group on 21st DPT showing a flattened layer of regenerating epithelium (E) covering the wound gap containing loosely arranged and disoriented collagen fibres (F_d).

H & E X 33

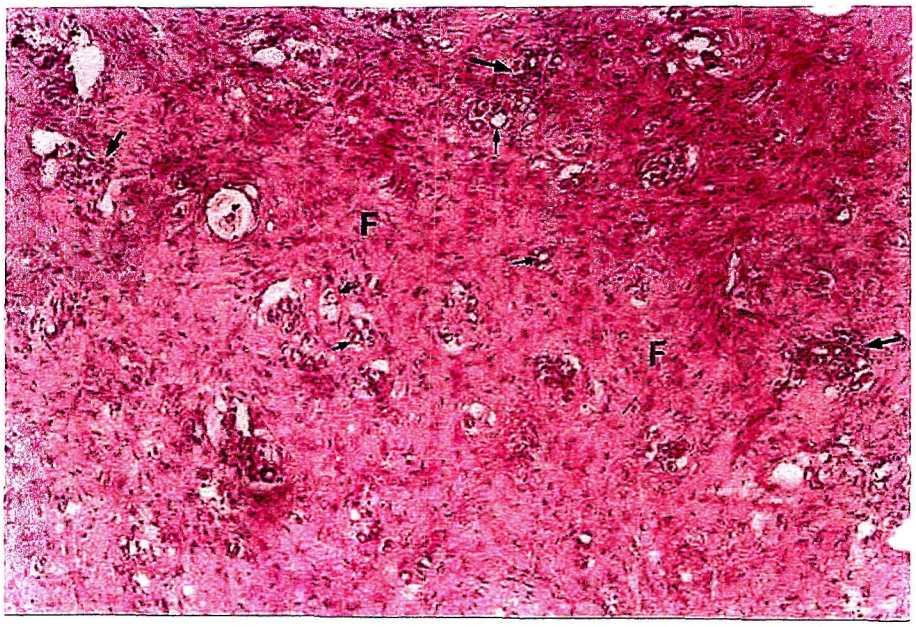


Plate-31: Photomicrograph from LPF group on 21st DPT from the same wound (plate-30) showing a large number of blood vessels and capillary loops (small arrows) along with leucocytes (big arrows) in between the fibrous tissue (F). **H & E X 33**

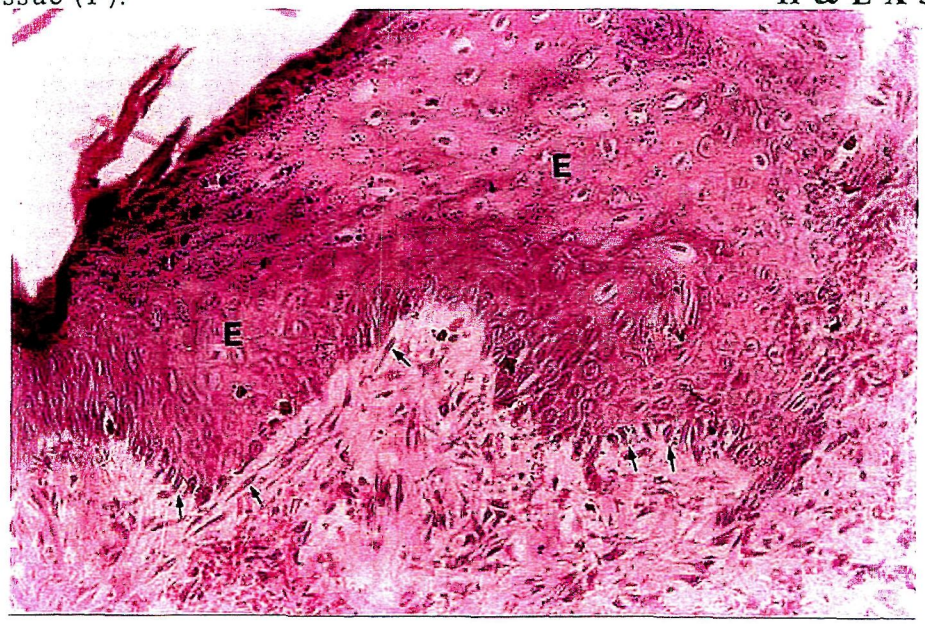


Plate-32: Photomicrograph from BET group on 21st DPT showing marked epithelial hyperplasia indicated by thickening and flattening of the epithelium (E). The basal cells (arrows) were seen separating out and migrating towards the areas of cell deficit.

H & E X 66

wound gap was completely filled by mature fibrous tissue which appeared somewhat disoriented at 21st DPT but was more organized at 28th DPT (Plate-34). The superficial parts of the wound appeared necrotic and infiltrated with leucocytes but epithelial regeneration was active beneath this necrotic layer which covered the granulation tissue (Plate-33). The tissues were richly collagenous at 28th DPT when sections were stained with vangeison's stain (Plate-34). Elastic fibres were absent in the healed tissue (Plate-35).

Seabuckthorn group:

The fibrous connective tissue appeared somewhat more organized and the collagen fibres were arranged parallel to skin surface both at 21st and 28th DPT (Plate-36). The granulation tissue was found completely replaced by the regenerating epithelium. The epithelial hyperplasia was a prominent feature. The healed tissue did not reveal either elastic fibres or normal adnexal structures of the skin but dense, organized collagen fibres were observed in vangeison's staining (Plate-37).

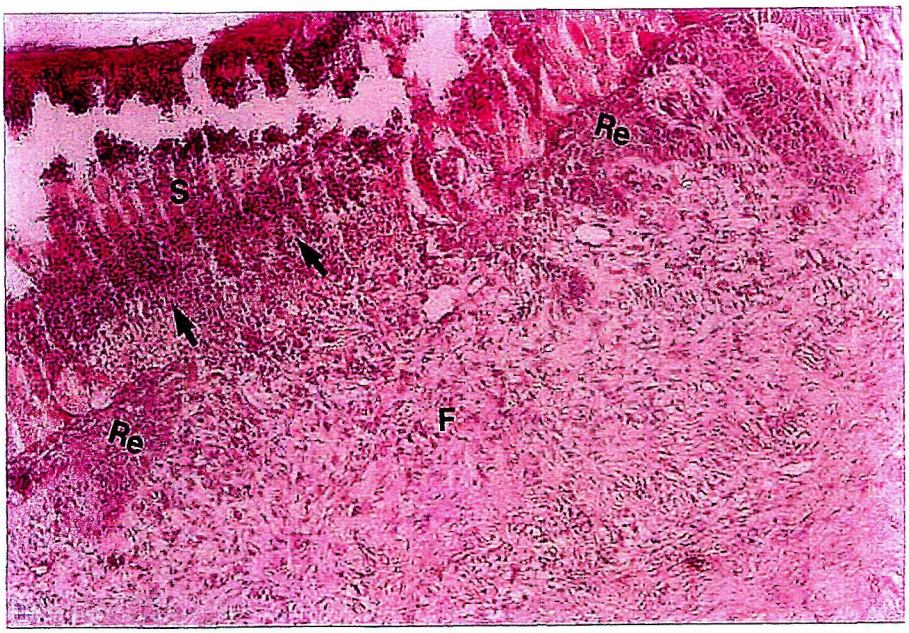


Plate-33: Photomicrograph from BET group from other animal on 21st DPT showing unorganized fibrous tissue(F) covered by the layer of regenerating epithelium(R_e) immediately below the superficial necrotic layer(s) infiltrated with neutrophils(arrows). H & E X 33

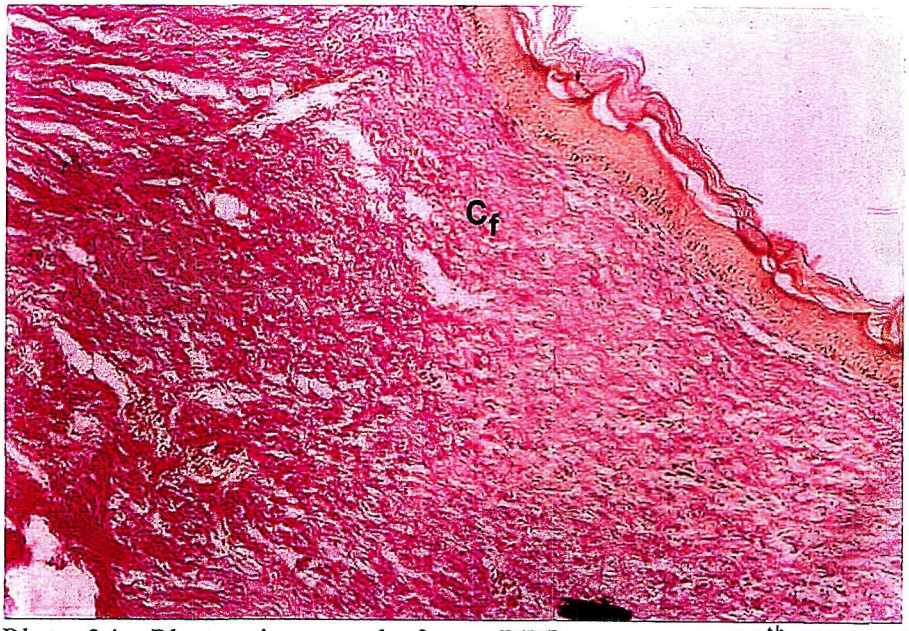


Plate-34: Photomicrograph from BET group on 28th DPT showing pink staining collagen fibres (C_F) completely filling the wound gap.

Vangeison X 33

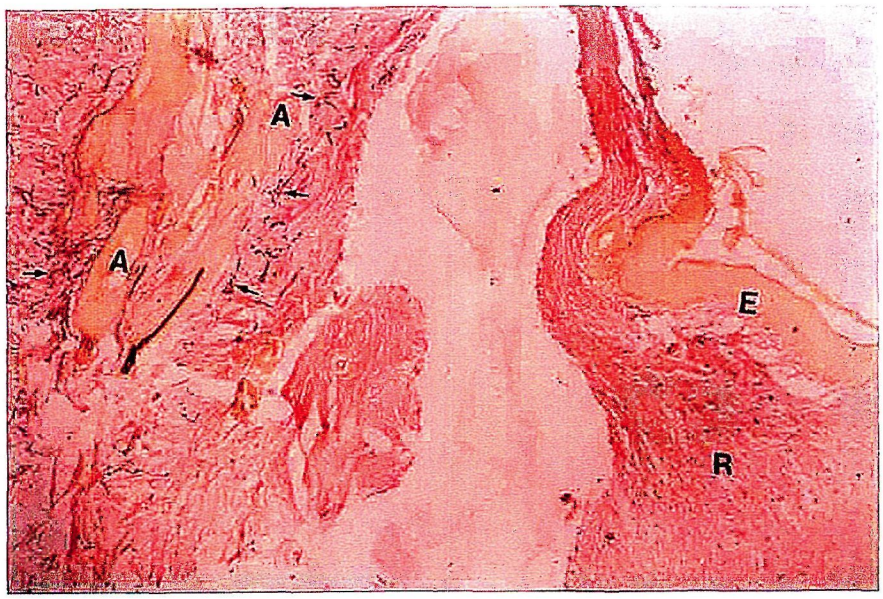


Plate-35: Photomicrograph from BET group on 21st DPT showing normal adnexal structures of the skin(A) along with black staining elastin fibres(arrows) towards the left side while latter were found absent in the healed tissue towards right side(R) which is covered by epithelium(E) Verhoeff X 13.2

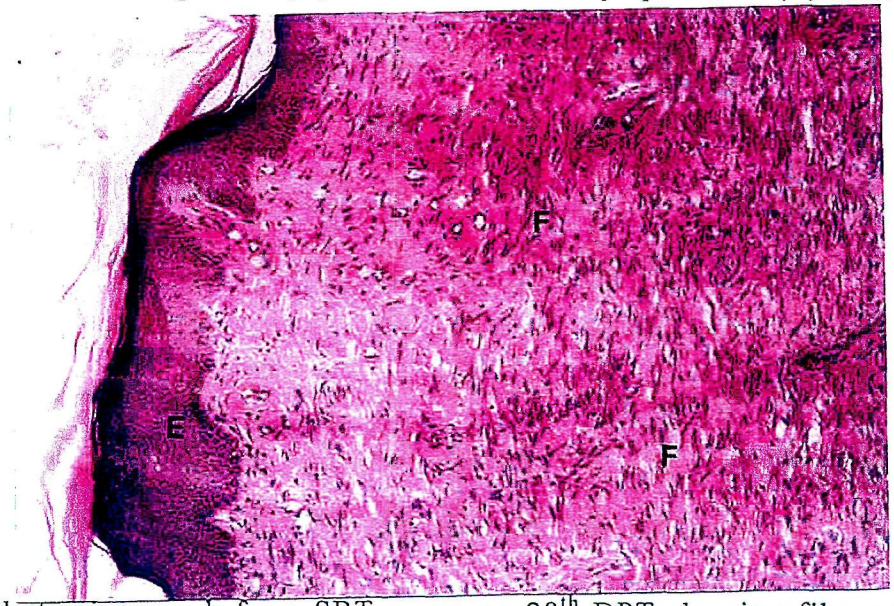


Plate-36: Photomicrograph from SBT group on 28th DPT showing fibrous tissue (F) appearing parallel to skin surface completely filling the wound gap and is covered by a layer of epithelium (E).

H & E X 33

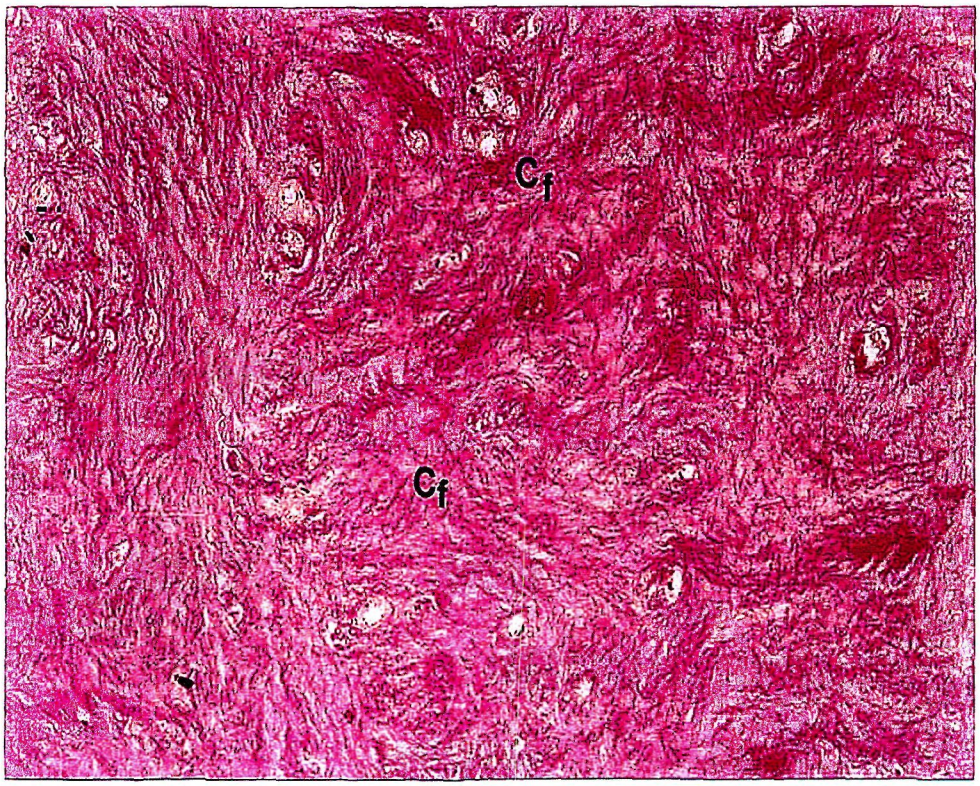
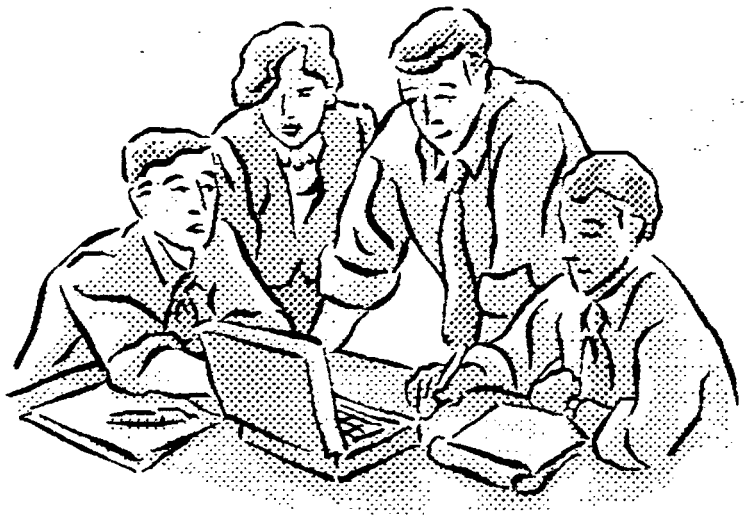


Plate-37: Photomicrograph from SBT group on 28th DPT showing pink staining organized bundles of collagen fibres (C_F) arranged almost parallel to the skin.

Vangeison X 33

DISCUSSION



DISCUSSION

Healing of the wounds is a dynamic and complicated process which is accomplished by the activity of a collection of cells that persue a unified goal. The process of healing has been divided into the phase of inflammation, cellular multiplication and collagen synthesis (granulation tissue), wound renewal and collagen maturation (scar tissue). These phases overlap and in fact, in maturation phase, new epidermis, fibroblasts and vessels are produced (Clark, 1993).

Seabuckthorn oil has wound healing properties (Vlasov, 1970; Mironov *et al.*, 1989; Buhatel *et al.*, 1991; Khirurgiia, 1995 and Gupta *et al.*, 2001). In the present study, the crude form of Indian variety of seabuckthorn i.e. fruit and seed were used after making their ointment in liquid paraffin(50% seabuckthorn ointment) to study the healing process of cutaneous wounds in dogs.

The oil contents in the fresh fruit varied from 4-8%, in fruit pulp 2-4% and seeds 10-20%.The pharmaceutical function of seabuckthorn oil involve the properties of antibacterial (Xu, Hanqing *et al.*, 1993), anti-inflammatory and regenerating tissue properties (Hou *et al.*, 1991). The phospholipids (0.5%) present in the ripe fruit help in cellular metabolism and hence in the healing process. Betaine in ripe fruit (0.09-0.36%) which is the methylating product of glycine has anti-ulcer and curative

properties. Similarly the flavonoids contained in the seabuckthorn oil have anti-inflammatory effects (Chen, Tigong *et al.*, 1988).

The triterpene components present in fruit oil (Ursolic Acid) is mainly responsible for wound healing, ulcer healing and in easing inflammation and pain (Ge, Xiaoyan *et al.*, 1986). The exact mechanism responsible for augmenting wound healing by seabuckthorn flavonoids present in its oil remain speculative. Free radicals are generated at the site of injury which impairs the healing process. Since seabuckthorn flavonoids have anti-oxidative effects so it is possible that healing activity of seabuckthorn might have been due to its potential to combat oxidative stress (Gupta *et al.*, 2001).

Clinical Signs:

The rectal temperature and heart rate remained with in the normal range throughout the period of observation in all the three groups. These observations in the present study simulate to the findings of Sharma and Bhardwaj (1995), Patel *et al.* (1999) and Bhardwaj and Sharma (1997) while studying the wound healing in different animal species using different medicaments.

A variation in the respiration rate was observed in between the groups as well as between the days during entire course of study. However in contrast to the results of present study, Sharma and Bhardwaj (1995), Patel *et al.* (1999) and Bhardwaj and Sharma (1997) reported no significant change in respiration rate during healing of different kinds of

wounds using different medicaments in different species. This might be due to the different physiological mechanisms operating in different animal species as well as variable response to different kinds of environmental and stress factors encountered during the creation of the wounds and subsequent therapeutic management of the wounds throughout the period of study.

The signs of acute inflammatory reaction i.e. rubor, calor, dolor, swelling varied from nil (-) to mild (+) in group-I and group-II animals as compared to the control group where the signs were of mild (+) to moderate (++) intensity. These signs remained in the control group up to 7^{th} day as compared to group-I and group-II animals where these signs were subsided by day 3^{rd} post treatment. This might be due to the anti inflammatory properties of seabuckthorn (Lebedeva *et al.*, 1989; Xu Mingyu *et al.*, 1993; Hou *et al.*, 1991).

However in contrast to these results, no visual signs of inflammation were reported in wounds treated for 7 days with seed oil and flavonoids of seabuckthorn in albino rats (Gupta *et al.*, 2001). This may be attributed to the fact that in the present study, fruit and seeds of seabuckthorn were used as such after grinding it whereas Gupta *et al.* (2001) used seabuckthorn oil which is having more anti-inflammatory properties. Secondly the size of the punched wounds was very small (8mm) in the previous study as compared to the size of wounds in the present study (1.5 X 1.5cm).

A mild (+) to moderate (++) degree of wound exudation was noticed on 3rd DPT in all the three groups. The wound exudation was observed even on 7th DPT in control group as compared to group I and group II. In contrast to these results, Gupta *et al.* (2001) reported marked dryness and no exudation through out the period of study while using seabuckthorn oil in healing of cutaneous wound in rats. This might be due to the more anti-inflammatory and healing properties of seabuckthorn oil as compared to its fruits. The wound exudation might be due to the cell mediated immune response which releases various kinds of cellular enzymes as advocated by Burk (1971).

However, the present study corresponds to the findings of Bhardwaj and Sharma (1997), Gupta *et al.* (1992), Parikh *et al.* (1996), Pradhan (1995) Varshney and Verma (1990), who reported the wound exudation during the healing of wounds in the initial stages by using different medicaments in various kinds of wounds in different species of animals.

The response to the granulation tissue appearance was better in seabuckthorn and betadine treated wounds as compared to control group. Complete epithelialization and scar formation was observed in cutaneous wounds between 14th and 18th DPT in the animals of group I and group II as compared to control group animals. The epithelialization was more intensive and occurred earlier in group I animals as compared to group II and III animals. Similar results have also been reported by

Khirurgiia (1995) while studying the effect of an extract of seabuckthorn (*H. rhamnoides L*) on the healing of experimental skin wounds in rats and he found intensive and earlier epithelialization in seabuckthorn treated animals as compared to other groups.

The animals of group I and group II showed higher wound contraction rate as compared to group III. On 7^{th} DPT the wounds contracted up to 36% and 32% respectively in group I and group II animals as compared to group III which showed only 17% wound contraction. The wound contraction was comparable in group I (75%) and group II (77%) animals on 14^{th} DPT but in group III, it remained low (69%). Complete healing of all the wounds occurred in all the animals of group I, II and III by 21^{st} DPT.

These findings simulate with the results of Gupta *et al.* (2001) who reported 17% and 37% of wound contraction rates with seabuckthorn seed oil and flavonoids respectively while studying the healing process of cutaneous wounds in albino rats which underwent treatment for seven days.

The wound contraction rate has also been studied using saliva in cutaneous wound healing in bovines (Varshney *et al.*, 1994) who reported significantly more wound contraction rate in saliva treated wounds in comparison to control group that is 7.2%, 16.2%, 41%, 62% and 80.8% at 3, 7, 14, 21, 28 post wounding days. In another study using Himax in wound healing in bovines the percent wound contraction

observed was 5.13%, 18.73%, 31.46%, 52.84% and 65.89% respectively at 3, 7, 11, 15 and 25th DPT (Varshney *et al.*, 1990). Similarly Neem ointment have been used as a potent wound healer (Bhardwaj and Sharma, 1997) which showed 13.60%, 40.90% and 85.45% wound contraction at 7th, 14th and 21st DPT in calves.

The above findings indicate better wound healing properties of seabuckthorn and betadine as compared to saliva, neem and himax as far as wound contraction rates were concerned.

No changes in the haematological parameters i.e. Hb, PCV, TLC, TEC and DLC were noticed in all the animals of group-I, II and III when compared to 0 day value and the values remained with in the normal range. This might be due to the fact that no systemic infection has occurred during the whole period of study.

Histopathological findings:

The early changes in cutaneous wounds on 3rd DPT in all the groups were characterized by an acute inflammatory phase with varying degree of fibrinopurulent exudation, congestion, haemorrhages and leucocytic infiltration chiefly with neutrophils. However in group-I animals, the response to inflammation and exudation was comparatively milder as compared to group-II and group-III animals. Besides this, fibroblastic proliferation was a feature noticed as early as 3rd DPT in group-I animals. Similar findings in the initial stages of wound healing have also been reported by Allgower (1956), Ross (1971), Varshney et al.

(1990) and Gupta et al. (1992) while studying the healing process in different animal species using different medicaments.

At 7th DPT, the inflammatory reaction in cutaneous wounds was more pronounced in the animals of group-III than in group-I and group-II. Besides appearance of fibroblasts, a large number of capillary loops were opened in the healing tissue both in group-I and II. The response to the treatment was comparatively better in the seabuckthorn group(group-I) where major portion of the wound was found replaced by the fibroblasts and deeper parts also exhibited mature fibrous tissue appearing somewhat more organized along with angiogenesis.

The response to the various treatments on wound healing was quite variable among the animals of group-I, II and III on 14th DPT. While in the seabuckthorn group, the majority of wound gap was found replaced by mature fibrous tissue with little inflammatory reaction, the inflammatory response was more pronounced with plenty of necrotic tissue along with little formation of granulation tissue chiefly comprising the young fibroblasts in betadine treated group. In contrast to these findings, group-III (LPF) animals showed varying degree of inflammatory reaction with granulation tissue containing a proportionately increase in the mature fibrous tissue with a decrease in the number of fibroblasts in the healing tissue.

A comparatively less pronounced inflammatory reaction in the seabuckthorn treated group in the present study might have been due to its

anti-inflammatory, anodyne and bacteriostatic properties. (Zhang-Wenlu et al., 1988; Hou et al., 1991; Lebedeva et al., 1989).

The depression in the connective tissue formation in group-II and group-III animals in the early stages of healing might be due to systemic factors inhibiting the fibroblastic proliferation (Carrel, 1921, 1930) or collagenolytic effect of cellular enzymes (collagenase) as reported by Dehaan et al. (1974). Seabuckthorn treated animals showed better wound healing response as compared to that of control animals. The better healing property of betadine as compared to control group at this stage corresponds to the findings by Mohammad et al. (2001). The better healing properties of seabuckthorn might be due to the various biochemical constituents (flavonoids, tri-terpenes, betaine, vitamins and mineral elements) present in its fruit and seed which enhances healing process(Mingyu et al.,2001).

The more inflammatory reaction in cutaneous wounds of group-II (Betadine) animals even up to 14^{th}- 21^{st} DPT might be due to the fact that betadine destroys fibroblasts and healing tissues (Lynne, 1999). It is reported that the use of betadine in wounds slows healing and causes injury and death of the tissue (Ollie, 1999).

Rodeheaver (1989) noted that not only the betadine is cytotoxic but that the wounds treated with betadine had an increased number of infections which result in more inflammatory reaction and delayed wound healing process.

The mild inflammatory reaction at 14th DPT in group-1 (seabuckthorn) animals along with the deposition of mature collagen fibres in the healing tissue is indicative of faster healing by the seabuckthorn ointment as compared to betadine and liquid paraffin. The faster healing process can be explained with rich contents of vitamins(A,C,E etc.) and microelements(S, Se, Zn, Cu etc.) in the seabuckthorn fruit along with the triterpene components which have regenerative and epitheliotropic properties(Xu Mingyu et al.,2001).

In the later stages(21st and 28th DPT), the fibrous connective tissue appeared somewhat more organized and the collagen fibres were arranged parallel to skin surface in group-I as compared to group-II and group-III animals where the mature fibrous tissue appeared loosely arranged and somewhat disoriented. The epithelial regeneration in cutaneous wounds was active in group-I and group-II animals as compared to control group. The epithelial hyperplasia was a distinct feature in all the three groups with varying activeness but leucocytic infiltration was still evident in the cutaneous wounds of group-II and group-III animals when compared with group-I (seabuckthorn) animals indicating delayed wound healing in group-II and group-III animals.

The healed tissue did not reveal either elastic fibres or normal adnexal structures of the skin (hair follicles, sweat glands, sebaceous glands etc.) in all the three groups. So at this stage, more collagenogenesis (maturation and contraction), capsularisation, granulation

tissue reaction particularly in the deeper zone along with complete epithelial covering was noticed in group-I(seabuckthorn) as compared to group-II(Betadine) and group-III(liquid paraffin) animals.

These studies also correlate with the findings of Khirurgiia (1995) who reported more intensive and earliar epithelialization, better and quicker granulation tissue differentiation (mature collagen fibres, profuse vascularity) during the healing of experimental skin wounds in rats with seabuckthorn extract over a 10 days period study indicating better healing properties of seabuckthorn. Similar results have also been reported by Varshney et al. (1990) and Sharma and Bhardwaj (1995), while studying the healing properties of Himax and Teeburb in bovines.

Histochemical studies:

The stainability of collagen fibres surrounding the blood vessels and fibroblasts increased at 7^{th} day onwards in cutaneous wounds of group-I and group-II up to 28^{th} day as compared to group-III where collagen fibres were detected in the later stages (Vangeison's stain) meaning that mature collagen fibres appeared earliar in group-I and group-II animals as compared to control group.

Similar observations have also been made by Gupta et al. (2001) who reported higher contents of hydroxyproline in the wounds treated with seabuckthorn seed oil and flavonoids as compared to control animals. Since hydroxyproline is an indicator of collagen fibre contents, higher the hydroxyproline level, more will be the collagen contents

indicating that the results in the present study simulate with the findings of Gupta et al. (2001).

Similar findings were reported while studying the efficacy of Curcuma- longa and Hellianthus-annus on tissue repair in calves in which significant higher contents of collagen and hydroxyproline were noted in the treated wounds as compared to their respective controls from day 7 to 28 (Tugnaiyat et al.,2000).

In the present study, after 7 days, the collagen fibres became coarse and started arranging themselves in the bundles in an effort to bridge up the wound gap in a direction parallel to that of stress. These findings simulate with the findings of Gibson and Kenedi (1967) and Jacques and Camerson (1969). The parallel arrangement of collagen fibres was more evident in cutaneous wounds of group-I animals as compared to that in group-II and group-III animals.

However the elastin fibres and normal adnexal structures of the skin were found absent in the healing tissue of cutaneous wounds throughout the period of study in the animals of all the groups.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted in nine adult healthy mongrel dogs of either sex (6 females and 3 males). The dogs were divided into three groups of three animals each. In all the animals, six full thickness excisional cutaneous wounds, three on either side of the vertebral column were created under general anaesthesia. The animals in group-I and group-II were treated by the topical application of 50 % seabuckthorn ointment and 5% betadine ointment respectively, whereas the animals in group-III (Control group) were treated by topical application of liquid paraffin up to 28 days.

All these animals were maintained on adlib diet (Bread/Chapattis, Eggs, Dalia, and Milk etc.) and water during the entire course of study. The parameters such as clinical and haematological observations, histopathological and histochemical studies were recorded at 0 and 3rd, 7th, 14th, 21st and 28th DPT (day post treatment).

The rectal temperature and heart rate in the animals of all the groups remained with in the normal range and did not show any significant change during the entire course of study; however respiration rate showed a significant difference in between the groups as well as between the days. Grossly the signs of acute inflammatory reactions in cutaneous wounds were less pronounced in seabuckthorn and betadine treated groups as

compared to control group and the signs in the later group remained up to 7^{th} DPT whereas these subsided by day 3^{rd} post treatment in seabuckthorn and betadine treated groups. The exudation in cutaneous wounds of the animals of group I and II subsided by day 7^{th} as compared to group III animals where it remained up to 7^{th} DPT with mild to moderate intensity.

The granulation tissue formation was noticed in cutaneous wounds on 3^{rd} DPT in seabuckthorn and betadine treated animals from the base as compared to control group where the granulation tissue appeared on 7^{th} DPT. The cutaneous wounds of group-I and group-II animals showed complete and extensive epithelialization and scar formation between 14^{th} –18^{th} DPT as compared to control group where complete scar formation was observed between 21^{st}-24^{th} DPT.

The seabuckthorn and betadine treated wounds showed uniform wound contraction up to 36% and 32% respectively on 7^{th} DPT as compared to control group where only 17% wound contraction was observed but on 14^{th} DPT the wounds contracted up to 75% and 77% respectively in seabuckthorn and betadine treated animals as compared to control group(69%).

The complete contraction of all the wounds was noticed at 21^{st} DPT in all the animals of group I, II and III with minor differences only. On comparative basis, A significant difference was noticed ($p<0.05$) in wound contraction rates in the animals of group-I(seabuckthorn) and group-II(betadine) when compared to control group(liquid paraffin) but no

significant difference was noticed in wound contraction rates between seabuckthorn and betadine treated animals.

No changes in the haematological parameters were observed in all the animals of all the groups during entire course of study. On the basis of clinical signs and gross findings, the wound healing appeared to be better in the animals of group-I and group-II as compared to group III.

Histopathologically, the response to inflammation and exudation was comparatively milder in the wounds of group-I animals as compared to group-II and group-III animals. Besides this fibroblastic proliferation was a distinct feature noticed as early as 3rd DPT (day post treatment) in the wounds of group-I animals.

The majority of the wound gap was found replaced by mature fibrous tissue with little inflammatory reaction at 14th DPT in seabuckthorn group as compared to betadine and liquid paraffin group. The inflammatory reaction in the cutaneous wounds of the animals of group-II was observed even up to 14th-21st DPT. The mild inflammatory reaction at 14th DPT in group-I along with deposition of mature collagen fibres in the healing tissue was indicative of faster healing in the seabuckthorn ointment treated wounds as compared to betadine and control group.

In the later stages, the fibrous connective tissue appeared somewhat more organized and the collagen fibres were arranged parallel to the skin surface in cutaneous wounds of group-I (seabuckthorn) as compared to group-II and group-III animals where the mature fibrous

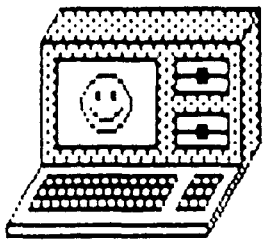
tissue appeared loosely arranged and disoriented. The epithelial hyperplasia was a distinct feature in all the wounds of animals of the three groups with varying activeness but leucocytic infiltration was evident in group-II and group-III animals when compared to group-I animals indicating delayed wound healing in group-II and group-III animals.

More collagenogenesis (maturation and contraction), Capsularisation, granulation tissue reaction particularly in the deeper zone along with complete epithelial covering in the later stages was a distinct feature in the wounds of animals of group-I as compared to group-II and group-III. Histochemically, mature collagen fibres appeared earliar in cutaneous wounds of group-I animals as compared to group-II and group-III animals as indicated by Vangeison's staining.

The parallel arrangement of collagen fibres was more evident in the wounds of animals of group-I as compared to group-II and group-III. However, elastin fibres and normal adnexal structures of the skin were found absent in the healing tissue throughout the period of study in all the animals of group-I, II and III.

The following conclusions can be drawn humbly on the basis of findings of present work:

1. No changes in the haematological parameters were observed during the healing process of cutaneous wounds of all the animals of group-I, II and III.
2. Seabuckthorn ointment seems to have better anti-inflammatory properties as indicated by mild inflammatory signs and exudation in cutaneous wounds of the animals of group-I as compared to group-II and group-III animals.
3. On comparative basis, the therapeutic efficacy of Seabuckthorn ointment in cutaneous wounds was found better than 5% betadine and liquid paraffin.
4. Being economical and having good soft tissue healing properties, seabuckthorn ointment can be used for dressing of cutaneous skin wounds in routine clinical cases of canines.



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