

Final Report

**National Agriculture Technology Project (CGP)
Indian Council of Agricultural Research, New Delhi**

Studies on the efficacy of Seabuckthorn (*Hippophae* sp.) on soft tissue repair with particular reference to cutaneous wounds, burns and gastric ulcers

Mode of research CGP

Code No. C-30892



Principal Investigator Dr. A. C. Varshney

Lead Centre

**Department of Surgery and Radiology
College of Veterinary and Animal Sciences
CSKHPKV, Palampur, 176062 (H.P.)**

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Executive summary

In this project the efficacy of Indian varieties of Seabuckthorn (*Hippophae rhamnoides*) was evaluated on aseptic and infected incisional and excisional cutaneous wounds, burns and gastric ulcers in animals. Seabuckthorn is known for its various medicinal properties since ancient times and is currently being commercially exploited in a big way mainly in China and Russia. However, in India, little attention was paid before this project to verify the pharmacological utilization of Indian varieties of this plant so as to improve the economics of any future cultivation of Seabuckthorn.

Before undertaking various kinds of research studies, potential toxicity of SBT berries and its oil was ruled out by various standard toxicities studies upon its oral administration as well as on its topical application in experimental animals. No clinical, haematological and biochemical adverse effects was observed in these studies. This indicates that oral administration and topical application of SBT fruit powder and oil is non-toxic.

The anti-inflammatory activity of seabuckthorn (SBT) seed oil on acute and sub acute inflammation was evaluated using subcutaneous injection of 5% Lugol's-iodine (0.1 ml) and cotton granuloma inflammation models respectively in dogs. Oral administration of moderate quantities of SBT oil (1 ml/kg) showed comparative anti-inflammatory activities as shown by 'acetylsalicylic acid', a routinely used drug in acute inflammation studies. However, the anti-inflammatory effect of SBT oil on sub-acute inflammation could not be established in the present model of study till 21 days.

In-vitro anti-microbial activity of SBT oil was evaluated by culture sensitivity disc method against *Staphylococcus aureus* and it showed mild to moderate zone of microbial growth- inhibition. However, in comparison to many sensitive broad spectrum antibiotics like ampicillin, ciprofloxacin and gentamicin etc, its zone of inhibition was relatively smaller. That indicates mild anti-microbial activities of seabuckthorn oil.

The efficacy of SBT oil in the healing of incisional cutaneous wounds was evaluated and compared with 5% povidone-iodine ointment and liquid paraffin in calves. The wound healing was studied on the basis of clinical, haematological and histopathological examinations at different intervals till 28 days. Clinically the SBT oil treated wounds remained relatively drier throughout the period of study and showed lesser inflammation. The increase in tensile strength of healing tissue was also found to be greater in SBT oil treated wounds at every stage of study.

The efficacy of SBT dried berry powder ointment was evaluated on aseptic excisional wounds in dogs and similarly compared with 5% povidone-iodine ointment (Positive

control group) and liquid paraffin (Negative control group). SBT ointment was found better than 5% Povidone-Iodine and liquid paraffin as indicated by lesser degree of inflammation, lesser wound exudation and faster percent wound contraction upon clinical examination at various observation intervals till 28 days. Histopathological examinations also revealed better granulation tissue response, early and greater collagen fibers synthesis and their better remodeling in SBT ointment treated wounds.

The effect of SBT dried berry powder ointment on the healing of *Staphylococcus aureus* infected cutaneous wounds was also evaluated and compared similarly in calves. This study revealed lesser inflammation and exudation in SBT and 5% Povidone-iodine treated wounds. Whereas, greater wound contraction in early stages was observed in SBT treated wounds. Histopathologically least inflammatory response was observed in Povidone-iodine treated wounds followed by SBT treated wounds in early stages. However, better wound healing in SBT treated wounds at later stages was achieved as evidenced by presence of relatively greater amount of mature fibrous tissue with complete epithelization.

Similar studies on the efficacy of SBT oil in cutaneous burn wound healing in calves proved better healing properties of SBT oil as revealed by early shedding of burn eschar, lesser inflammatory reactions and more organized healing tissue in this group.

The therapeutic and prophylactic efficacy of seabuckthorn oil in experimentally created gastric erosion/ulcers in dogs was evaluated by clinical, haematological, biochemical, radiological, endoscopical and histopathological examinations. These were compared with 'Omeprazole'-treated positive control and untreated negative control groups. The group of animals that were fed seabuckthorn oil prophylactically showed lesser intensity of gastric erosions endoscopically in response to ulcerogenic drugs as compared to other animals. Clinically also these animals did not exhibit the usual accompanying symptoms of gastric erosion/ulceration such as loss of appetite, vomiting and melema etc. The animals treated with seabuckthorn oil after development of gastric erosions/ulcers also exhibited earlier disappearance of gastric lesions endoscopically as compared to untreated group.

It is therefore concluded that seabuckthorn has a therapeutic potential for the management of various kinds of aseptic and infected cutaneous wounds, burns and gastric ulcers in animals. However, more studies are needed to identify the various bio-active ingredients of the SBT plant useful in above mentioned properties so that better utilization of plant component may be undertaken.

- 1 **Title:** “Studies on the efficacy of Seabuckthorn (*Hippophae* sp.) on soft tissue repair with particular reference to cutaneous wounds, burns and gastric ulcers”
- 2 **Code No.** C-30892
- 3 **Mode of research:** CGP
- 4 **Name of Agro-ecosystem:** -
(For PSR projects only)
- 5 **Name of P.I.** Dr. A.C. Varshney, Prof. and Head Dept. of Surgery & Radiology, COVAS, Palampur
- Co-Principle Investigators:**
- Dr. SP Tyagi, Assistant Professor, Dept. of Surgery & Radiology, COVAS, Palampur. (32 years, Male)
 - Dr. VK Gupta, Associate Professor, Dept. of Pathology, COVAS, Palampur (42 years, Male)
 - Dr. Virendra Singh, Assistant Scientist, Dept. Of Agroforestry, COA, Palampur (43 years, Male)
- 5 **Date of start of the sub-project** 15-07-2002
- 6 **Date of termination of the sub-project** 31/12/2004
- 7 **RA employed with details of their engagement period (In tabular form with age and sex)**

| Name | From | To | Age | Sex |
|------------------|------------|-------------|-----|------|
| Dr. Munish Gupta | 07/09/2002 | 02/05/62003 | 30 | Male |
| Dr. Amit Kumar | 25/07/2003 | 20/12/2004 | 26 | Male |

9 **Brief financial status:**

| | |
|------------------------------------|-------------------------|
| Original Sanctioned Amount: | Rs. 20,72,870.00 |
| Additional sanctions: | Rs. 3,86,298.00 |
| Actual expenditure: | Rs. 23,12,940.00 |

Introduction:

Wound healing is a complex and dynamic process, which requires maintenance of optimal environment for its natural progress. The world over research is continuously being pursued to find newer and better wound dressing means. One of the big focus of such research is to develop plant based medicines due to their diverse mechanisms of action. Seabuckthorn is one such plant already widely acclaimed for its various medicinal properties. In India, the abundant quantities of seabuckthorn grow wildly in Himalayas but remain largely unutilized and are of no great use to local population. The present project was aimed to verify the soft tissue healing properties of Indian varieties of seabuckthorn so that this plant may be utilized by pharmaceuticals.

Objectives:

- ❖ To study the toxicity of seabuckthorn in experimental animals.
- ❖ To study the anti-inflammatory activities of seabuckthorn in animals.
- ❖ To study *In-vivo* and *In-vitro* anti-microbial activities of seabuckthorn.
- ❖ To study the effect of seabuckthorn on the healing of incisional and excisional aseptic wounds in animals.
- ❖ To study the effect of seabuckthorn on the healing of infected cutaneous wounds in animals.
- ❖ To study the effect of seabuckthorn on healing of experimentally induced full thickness burns in animals.
- ❖ To study the efficacy of seabuckthorn on experimentally induced gastric ulcers in animals.

Significant achievements:

Before undertaking animal experimentations for various studies of this project, the necessary approval was duly obtained from appropriate authority.

1 Studies for the evaluation of potential toxicity of Seabuckthorn:

a. **Studies upon oral feeding of Seabuckthorn in animals:** The present study was conducted in nine adult healthy mongrel dogs of either sex weighing between 10-18 Kg divided into three groups of three animals each. Seabuckthorn (SBT) fruits along with seeds were dried, finely grounded using grinder and cyclotone mill and were fed @ 5 grams and 10 gms per day to each animal of group I and II respectively for 21 days. Whereas, group III served as untreated control in which no SBT powder was fed. The development of any kind of toxic effect was monitored by clinical, haematological and biochemical examinations at different time intervals at 0, 3, 7, 14 and 21 days.

Results:

The rectal temperature, respiration rate and heart rate in all the animals of three groups remained within the normal range and did not show any significant change during entire course of study. Feed intake was also normal in all the animals of all groups with no change in the body weight throughout the period of study. No significant change was observed in the haematological parameters throughout the period of study in all the animals of three groups. Although group II and group III animals showed slight neutrophilia from 0 day onwards to 21 days post feeding but the increase was not statistically significant ($P > 0.05$). Blood glucose and total plasma proteins of the animals also remained within normal physiological range. Blood urea nitrogen (BUN) levels declined from 0 day to 7th day and thereafter increased gradually but non-significantly up to 21 days as compared to control group. The plasma creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) levels showed non-significant increase from 0 day onwards up to 21 days in all the three groups.

These results prove that oral feeding of seabuckthorn berries in optimum dosage are safe and non-toxic.

b. Studies upon topical application of Seabuckthorn oil in animals:

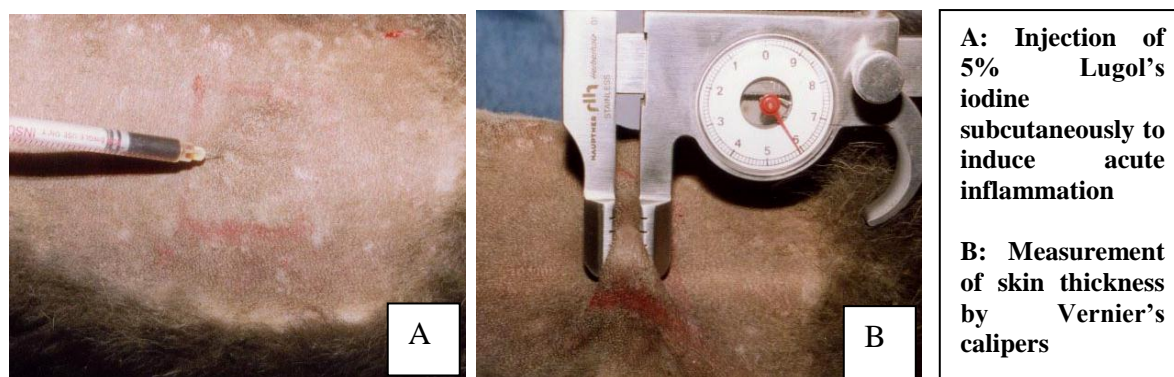
For this study, five dogs of either sex were utilized. Their dorso-lateral lumbar region was shaved and two equidimensional area of 2X2 cm were outlined on either side of the vertebral column. The SBT seed oil derived by ether extraction method was then applied once daily on the skin at these specified area with a sterile gauge piece continuously for 15 days. This gauge piece was left adhered to the skin with the help of a

millipore paper tape for at least 12 hours. The development of any kind of toxic effects or irritation due to SBT seed oil was observed clinically. The oil did not produce any kind of toxic effects on the skin. It proved that Seabuckthorn oil is non-irritating and is not toxic upon its topical application.

2 Studies for the evaluation of anti-inflammatory activities of Seabuckthorn:

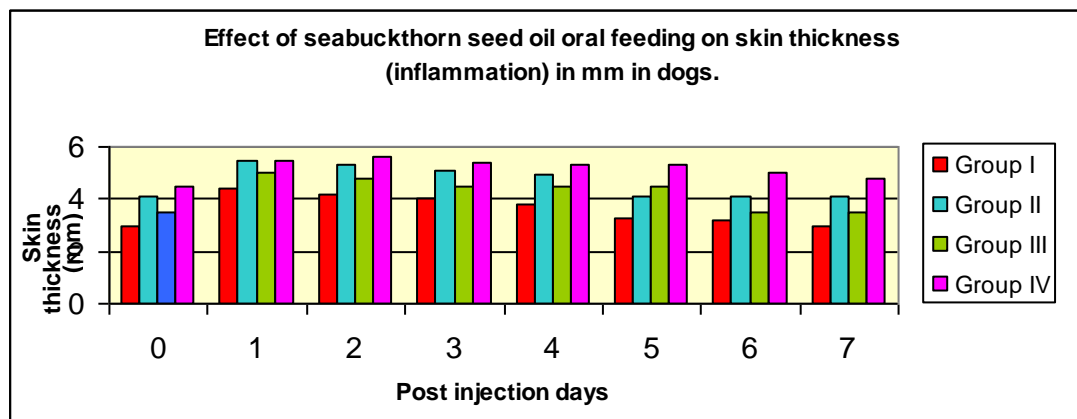
a) Studies for evaluation of the effect of Seabuckthorn on acute inflammation in experimental dogs: The study was conducted in twelve dogs of either sex randomly divided into four groups (Group I, II, III and IV) of three animals each. The dorsolateral lumbar region was prepared as for aseptic surgery and one 2X2 cm area on its right side was outlined. In all these animals, acute inflammation was developed at the specified area by injecting 0.1 ml of 5% Lugol's Iodine sub-cutaneously. Seabuckthorn seed oil was orally fed @ 0.5 and 1.0 ml/Kg body weight once daily to the animals of group I and II respectively for seven days. The animals of group III were fed with Acetylsalicylic Acid dispersible tablets @ 20 mg/Kg body weight once daily till inflammation subsided, whereas the group IV served as untreated negative control. The development of inflammation and anti-inflammatory effects was monitored by clinical symptoms and skin thickness measurement in the marked area by Vernier Calipers at various time intervals. Statistical analysis of the data was carried out using analysis of variance (ANOVA) and comparison between the treatments and days was done at 5 % level of significance.

Results: The sign of acute inflammation i.e. swelling, erythema, warmth was observed as early as 2 hours after injection of 0.1 ml of 5% Lugol's iodine in all the four groups of animals which is further confirmed by skin thickness measurement using vernier calipers.



The signs of acute inflammation as well as skin thickness started decreasing from day 1 onwards in groups I, II and III whereas in group IV it was observed 3rd day post injection. The skin thickness decreased to normal by day 5th, 6th and 7th in group II, III and I

respectively whereas in group IV, it took even longer. The results thus indicated anti-inflammatory properties of seabuckthorn oil upon oral feeding in dogs.



b) Studies for evaluation of the effect of Seabuckthorn on sub-acute inflammation in experimental dogs:

The study was conducted in 12 dogs of either sex randomly divided into four equal groups (I, II, III and IV). In this study, 4 sterile cotton pellets each weighing 100 ± 5 mg were inserted aseptically under general anaesthesia subcutaneously in the dorsal thoracolumbar region of all the animals at four different places to produce subacute inflammation. The site was covered with sterile gauze fixed with adhesive tape followed by a second layer of clean cloth. The dressings were changed once daily till 10th day and on alternate days thereafter. The animals of group I and group II were fed seabuckthorn oil orally @ 0.5ml/kg and 1ml/kg body weight respectively once daily. In group III seabuckthorn oil was applied topically over the cotton pellet insertion site whereas group IV was kept as untreated control. Treatment was continued for 21 days in all the groups. The development of subacute inflammation and its progression were monitored by routine clinical examinations and the weight of cotton granuloma at different intervals. The cotton pellets along with granuloma formed around them were removed aseptically under anaesthesia one at a time on day 3, 7, 15 and 21 of study. The pellets obtained were dried in oven overnight and their weight recorded. The weight of granuloma was ascertained by subtracting the weight of cotton pellet (100 mg) from it.

Results: Rectal temperature, respiration rate, heart rate did not show any significant change at different time intervals. Purulent exudate was observed in almost all the pellet insertion sites from 7-9 days onwards in all the groups which however subsided after removal of cotton pellets at different time intervals. The gross clinical symptoms of inflammation such as redness, localized irritation and warmth were comparable in all the

groups throughout the study. The weight of cotton granuloma did not show any significant difference between the groups at different time interval. It is thus concluded that the anti-inflammatory effect of SBT oil on sub-acute inflammation could not be established in the present model of study till 21 days.

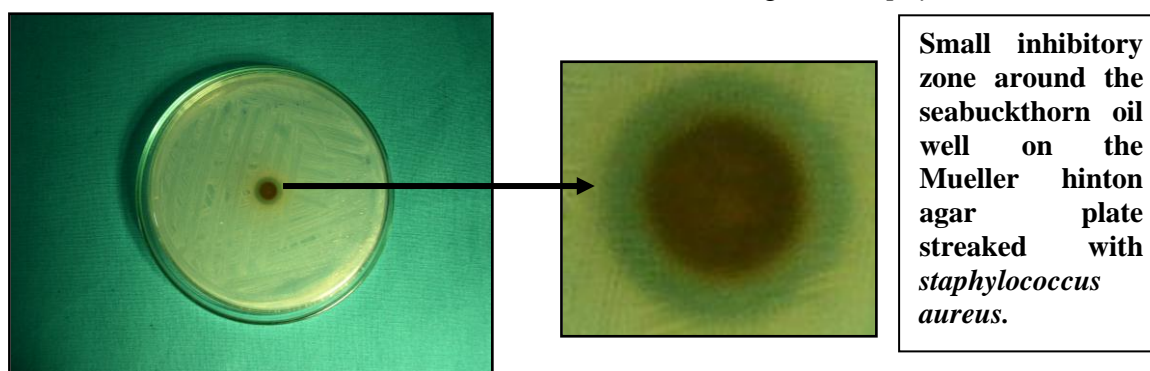
Table 1: Weight of cotton granuloma at different time intervals

| | 0 day | 3 rd day | 7 th day | 14 th day | 21 st day |
|-----------|-------|---------------------|---------------------|----------------------|----------------------|
| Group I | 100±5 | 106.66±13.33 | 132.66±12.47 | 143.66±12.99 | 147.33±13.67 |
| Group II | 100±5 | 98.33±19.22 | 123.33±12.02 | 138.66±11.83 | 143.66±12.86 |
| Group III | 100±5 | 130±30.00 | 145.66±20.28 | 152.33±22.25 | 156±22.31 |
| Group IV | 100±5 | 123.33±17.63 | 131.66±11.66 | 138.33±10.14 | 143±10.12 |

3. Studies for the evaluation of anti-microbial activity of Seabuckthorn

In-vitro studies: Culture sensitivity test of SBT oil was performed on Mueller-Hinton agar by preparing wells. Then SBT oil mixed with DMSO was filled in these well and sealed routinely. The agar plate was then streaked with *Staphylococcus aureus* and incubated at 37°C for 12 hrs. The antimicrobial activity of SBT oil was ascertained by evaluating the bacterial growth inhibition zone around these wells and compared with routine antibiotic's induced inhibitory zones.

Results: Small inhibitory zones around the SBT oil wells were observed after 12 hours of incubation period. However, in comparison to many sensitive broad spectrum antibiotics like ampicillin, ciprofloxacin and gentamicin, its zone of inhibition was relatively smaller. That indicated mild anti-microbial activities of SBT oil against *Staphylococcus aureus*.



4 Studies for the evaluation of wound-healing-promoting activities of Seabuckthorn on full thickness incisional wounds:

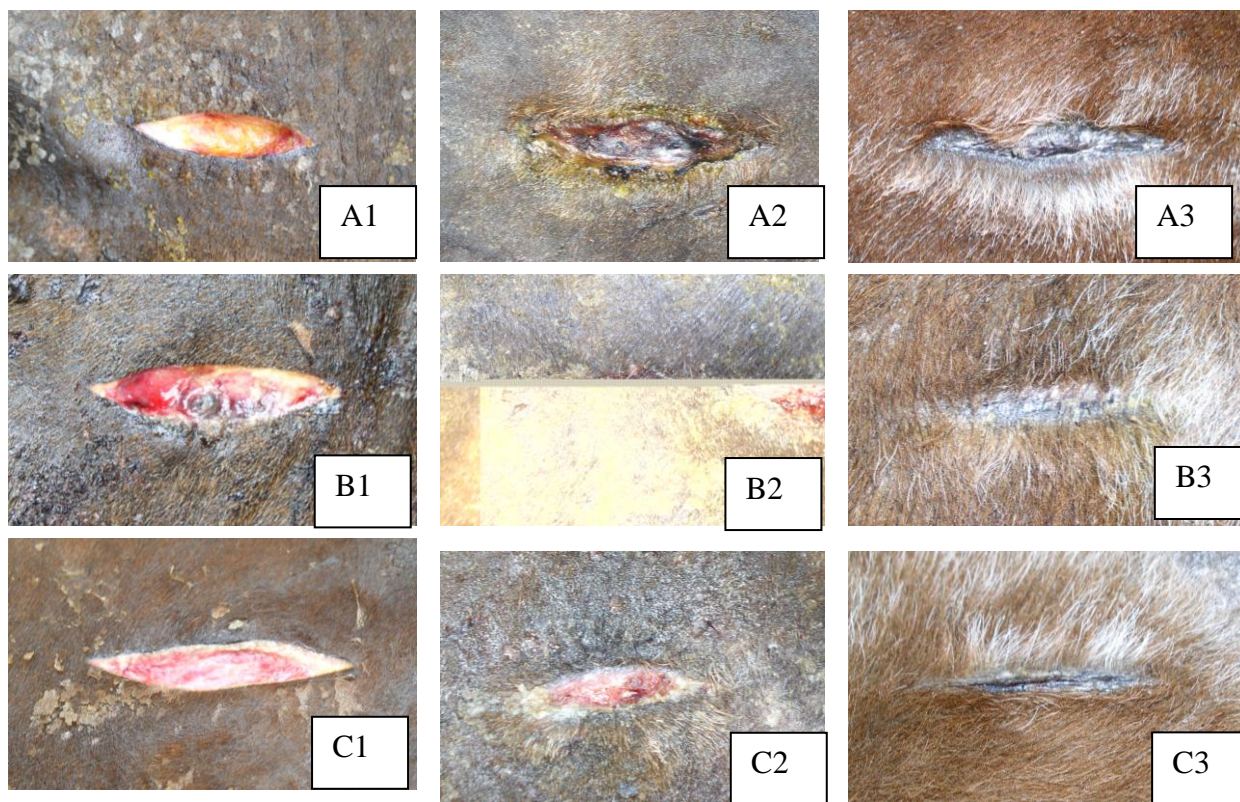
The study was conducted on nine 6-12 months old male calves, divided into 3 equal groups viz. I, II and III. Six, 2 inch long incisional full thickness wounds, 3 on either side of the vertebral column were created aseptically under local infiltration

anaesthesia on the dorsal thoracolumbar region of all the animals. The wounds were dressed once daily with liquid paraffin, 5% Povidone-Iodine ointment and SBT seed oil in group I, II and III respectively. Three trials were conducted to carry out the present research work and in each trial one animal from each group was taken. The wound healing was evaluated by clinical, haematological, wound tensile strength and histopathological observations at different time intervals. In clinical observations, rectal temperature, respiration rate, heart rate, degree of inflammation and gross appearance of wounds were recorded at 0 and 3rd, 7th, 10th, 15th, 21st and 28th day post-operatively. The degree of inflammation was categorized as nil (-), mild (+), moderate (++) and severe (+++). For haematology, about 0.5ml of venous blood was collected at above mentioned intervals and was analyzed for estimation of haemoglobin (Hb), packed cell volume(PCV), total erythrocyte count(TEC), total leucocyte count(TLC) and differential leucocyte count(DLC) using standard methods. The healing tissue strips of 1.5X1.0 cm were collected on these intervals for estimation of tissue tensile strength. 1.0X1.0 cm area of these tissue strips were affixed in a custom made tensiometer and its breaking strength were recorded. The tissue breaking strength at day zero was considered 100% and the subsequent healing tissue breaking strength were compared to this zero day value in terms of gain in strength in percentage. The values were then also subjected to statistical analysis between the groups. Similarly healing tissue biopsies were collected at these intervals under local infiltration anaesthesia from one wound at a time. The biopsies were processed using standard methods and their sections were stained with haematoxyline and eosine for routine histopathological examination. Special staining techniques like Vangeison's for collagen fibres, Verhoeff's for elastic fibres were used for histochemical studies. Statistical analysis of the data was carried out as described earlier.

Results:

The rectal temperature, respiration rate and heart rate of animals of all the groups remained within the normal range during the entire course of study and the variation in these parameters was non-significant. There was no clinically manifested complication throughout the study and animals remain active, alert and healthy. These findings imply that the topical application of SBT oil on the incisional wounds in calves was safe. Grossly the degree of inflammation in wounds was less pronounced in group III and II. In these groups the signs of inflammation starts subsiding by 3rd day but in group I these subsided by 10th day. All the animals of group I and one animal of group II showed wound exudation on day 3. Wound exudation continued upto 7th day in group I but

subsided in group II by 5th day. However no wound exudation was observed in group III at any stage implying that seabuckthorn oil possesses good anti-inflammatory property.

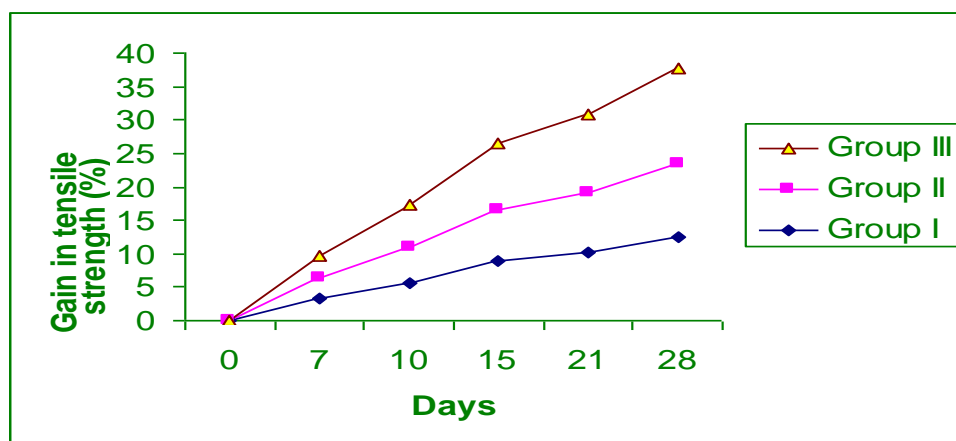


Gross appearance of the full thickness incisional wounds in calves treated with

Liquid paraffin (A1-3rd day; A2-15th day; A3-28th day), 5% povidone iodine (B2-3rd day; B2-15th day; B3-28th day, SBT seed oil (C1 oil-3rd day; C2-15th day; C3-28th day)

- **Photographs showing comparative early healing, faster wound contraction and epithelization in the seabuckthorn seed oil treated wounds in calves.**

Continuous gain in healing tissue tensile strength was observed from 3rd day onwards in all groups. However the gain in tensile strength was greater in SBT treated wounds at every observation intervals except on day 7, where it was marginally comparable to group I.



On 28th day the total gain in tensile strength was 12.55%, 10.92% and 14.28% in liquid paraffin, 5%povidone-iodine and seabuckthorn treated group respectively. There were no

significant changes in the haematological parameters i.e. Hb, PCV, TLC, TEC and DLC in any of the groups at different time intervals. All the values remain within normal physiological range. Histopathologically on day 3, the signs of inflammation were comparatively less in group III. The amount of ground substance in deeper parts of the wounds in group III was substantially greater at 7th day compared to other groups. At day 10 also, group III showed relatively more mature granulation tissue and in further later stages their collagen fibers were arranged parallel to skin surface and were much more organized. Whereas in other groups, the polymorphonucleocytes (PMN) infiltration in the wound surface was relatively greater in early stages and the orientation of collagen fibres was haphazard even at later stages.

These observations indicated that the seabuckthorn oil is superior to 5% Povidone-iodine ointment and liquid paraffin as evidenced by earliest disappearance of inflammation and highest gain in tensile strength of healing tissue.

5 Studies for the evaluation of wound-healing-promoting activities of Seabuckthorn on full thickness aseptic excisional wounds:

The present study was conducted in nine adult healthy mongrel dogs of either sex. The dogs were divided into three equal groups viz. I, II, and III. Six equi-dimensional (1.5 x 1.5 cm) full thickness excisional cutaneous wounds, three on either side of the vertebral column were created in all the animals on their dorsal thoracolumbar region aseptically under general anaesthesia. Wounds of all the animals were immediately subjected to differential wound dressings as per the groups. The wounds were dressed once daily with SBT ointment, 5% Povidone-Iodine ointment and liquid paraffin in group I, II and III respectively. SBT 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 SBT powder was then mixed with 100 ml of liquid paraffin to form 50% SBT ointment. The wound treatment was done daily till 28th postoperative day. Wounds were covered with a sterilized gauge over the dressing material secured with an adhesive tape. Another secondary protective covering of sterilized drapes encompassing all the wounds were also kept tied with the animal's body to prevent external contamination of the wounds. Moreover, to prevent licking and biting at the wound site by the animals, they were restrained by using neck collars and muzzle piece. Wound healing of the animals was evaluated on the basis of clinical, haematological and histopathological studies at 0, 3rd, 7th, 14th, 21st and 28th day. All the clinical observations as described in above study were recorded on these

intervals. Besides percent wound contraction was also measured by using the following formula:

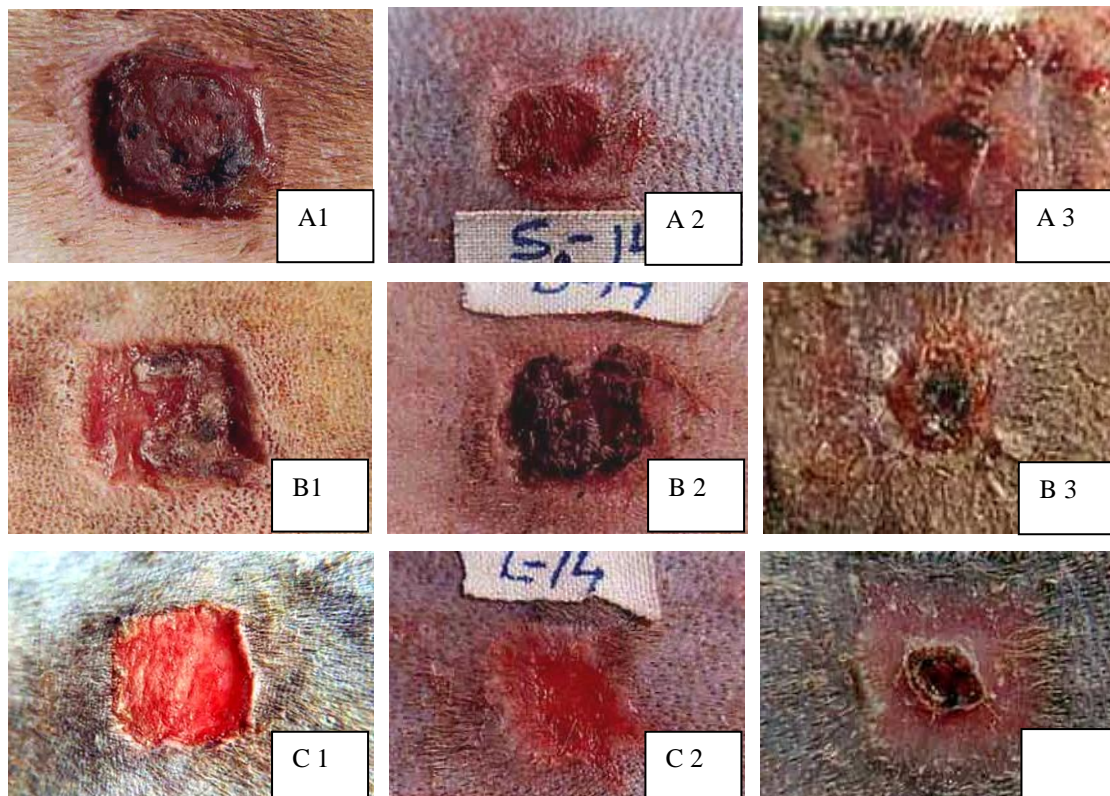
$$\text{Percent wound contraction} = (B-A)/B \times 100$$

Where B is the area (sq. cm.) of wound at day 0 and

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

For haematological and histopathological studies, same procedures were used as described in previous study. Besides, swabs were collected from healing wounds and subjected to culture sensitivity test (CST) at 3rd, 7th, 14th, 21st days to rule out the development of any infection. Statistical analysis was also performed as described above.

Results: The rectal temperature and heart rate remained within the normal range and almost constant throughout the period of observations in all the groups. But the respiration rate showed a significant difference ($P < 0.05$) in between the groups as well as between the days. However, all these fluctuations in the respiration rate remained within the wider normal physiological range and were not found reflective of any complication in wound healing. The signs of acute inflammatory reactions i.e. swelling, erythema, warmth varied from nil to mild in seabuckthorn and povidone-iodine treated groups as



Gross appearance of the full thickness aseptic excisional wounds in dogs treated with SBT ointment (A1-3rd day; A2-14th day; A3-21st day), 5% povidone iodine ointment (B2-3rd day; B2-14th day; B3-21st day), Liquid paraffin (C1 -3rd day; C2-14th day; C3-21st day)

- **Photographs of Seabuckthorn ointment treated wounds showing earlier filling of wound cavity by granulation tissue, faster development of scab and greater wound contraction.**

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 3rd whereas by 7th day in the control group. Mild to moderate wound exudation was noticed on 3rd day in all the three groups; it almost subsided by 7th day in group-I and II and by 11th day in group III. SBT and 5% Povidone-iodine treated wounds showed significantly greater contraction up to 36% and 32% respectively as compared to control group which showed only 17% wound contraction on 7th day. Though on day 7, the wound contraction in group I was greater than group II, however it was statistically non significant. Later on at day 14, the percent wound contraction was again greater in group-I and II i.e. 75% and 77% respectively, whereas it was considerably lesser in group III (69%).

Table: 2 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 operative days | | | | |
|----------|-----------------------|---------------------|-----------------------------|----------------|----------------|-----------------|
| | | 3 | 7 | 14 | 21 | 28 |
| I(n=3) | 0.00 | 14.33 ±11.14 | 35.98 ±6.03 | 75.83 ±4.27 | 97.92 ±0.29 | 100.00 ±0.00 |
| II (n=3) | 0.00 | 8.26 ±8.26 | 32.73 ±9.52 | 77.73 ±8.86 | 96.13 ±2.62 | 100.00 ±0.00 |
| III(n=3) | 0.00 | 6.66 ±6.63 | 17.01 ^A ±7.45 | 69.76 ±1.76 | 93.72 ±2.88 | 97.87 ±0.17 |

A indicates significant difference ($P<0.05$) in between the groups
n = number of animals.

No significant difference was found in PCV, DLC and TLC in between the groups at different time intervals. A significant difference was observed in the Hb and TEC values in between the groups but no significant change was seen in between the days in all the three groups when compared to day 0. Histopathologically in the initial stages of healing, the response to inflammation and exudation was comparatively milder in the wounds of group-I as compared to other groups. Besides, the fibroblastic proliferation was a distinct feature noticed as early as 3rd day in the wounds of group-I animals. The majority of the wound gap was found replaced by mature fibrous tissue with only little inflammatory reaction at 14th day in this group.



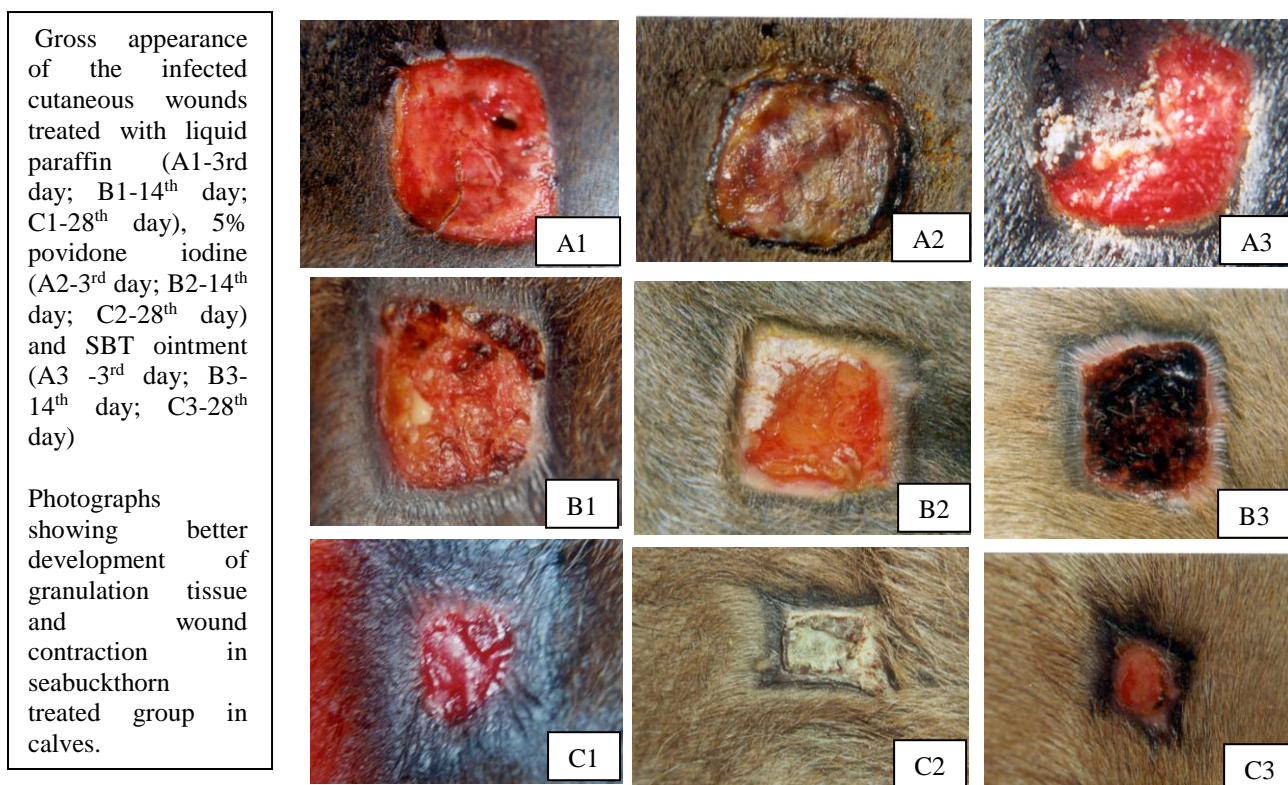
- A) Photomicrograph from SBT group on 14th day showing superficial necrotic tissue infiltrated with neutrophils and degenerated cellular elements (S) covering the mature fibrous tissue (F) which appeared more densely packed and organized.
- B) Photomicrograph from SBT group on 28th day showing fibrous tissue (F) appearing parallel to the skin surface completely filling the wound gap and is covered by a layer of epithelium.
- C) Photomicrograph from SBT group on 28th day showing pink staining organized bundles of Collagen fibres (C_F) arranged almost parallel to the skin.

Whereas, inflammatory reaction in the wounds of group-II and III animals persisted even up to 21st day. In the later stages, the fibrous connective tissue appeared somewhat more organized and the collagen fibres were arranged parallel to the skin surface in wounds of group-I when compared to other groups where the mature fibrous tissue appeared loosely arranged and disoriented. The leucocytic infiltration was greater in group-II and III wounds. On comparative basis more collagenogenesis (maturation and contraction), capsularisation, granulation tissue reaction particularly in the deeper zone along with complete epithelial covering in the later stages was observed in the wounds of group-I animals. It is thus proved that the seabuckthorn ointment is superior to 5% Povidone-iodine ointment and liquid paraffin as indicated by lesser degree of inflammation, lesser wound exudation and faster percent wound contraction in this group.

6 Studies for the evaluation of wound-healing-promoting activities of Seabuckthorn on full thickness infected cutaneous wounds:

The present study was conducted on 9 male calves divided into 3 equal groups namely group I, II and III. Six equidimensional (3cmx3cm) full thickness cutaneous wounds, 3 on either side of the vertebral column were created aseptically under local infiltration anaesthesia on the dorsal thoracolumbar region of all the animals. Thereafter, 1ml of *Staphylococcus aureus* suspension having 2.1×10^9 organisms/ml was injected subcutaneously into the wounds. The wounds were covered by NSS soaked sterile cotton gauze taped to site and further overlapped by a clean cloth. The infection was allowed to flare up for 48hrs and then differential wound dressings according to the groups were started. The wounds were dressed once daily with, liquid paraffin, 5% Povidone-Iodine ointment and SBT ointment in group I, II and III respectively Dressing of the wounds was done daily for 7 days and on alternate days for the next three weeks. The methodology of wound healing evaluation remained same as described in previous studies.

Results: The rectal temperature, respiration rate and heart rate remained within normal physiological range in all the groups throughout the period of study. Haematology revealed significant decrease in PCV along with non significant reduction in Hb in all the groups. Slight neutrophilia was observed following creation of infected wounds but overall TLC and TEC remained within normal range. Inflammation subsided by 7th day in groups II and III but by 14th day in group I. In all the groups, the wounds remained grossly purulent up to 7th day even after the start of the treatments. However, *Saphylococcus aureus* could be isolated from the site up to 14th day, irrespective of the treatment. Wounds in group II and III showed expansion till day 3 followed by

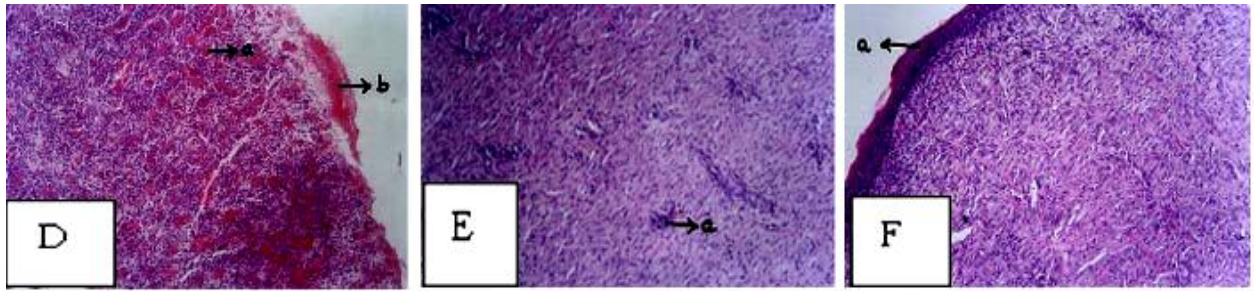


contraction, whereas group I wounds did not show any expansion clinically. The wounds contracted up to 50 % by day 14 and around 90 % by day 28 irrespective of the treatment. Wound contraction was comparable in all the groups after 7rd day but on comparative basis it was more in Groups I and III. Thereafter no significant difference in wound contraction with respect to different treatments was observed. However, a greater wound contraction was observed at day 21st in Group III in comparison to the other two groups.

Table: Effect on wound contraction (%) following different treatments of infected cutaneous wound in cow calves (Mean \pm SE).

| TREATMENT GROUPS | DAYS FOLLOWING TREATMENT | | | | | | |
|---|--------------------------|----|---------------------|---------------------|---------------------|---------------------|---------------------|
| | BEFORE INFECTION | 0 | 3 | 7 | 14 | 21 | 28 |
| PARAFFIN (GROUP I, n = 3) | ----- | -- | 0.47 ± 4.43 | 21.65 ± 3.54 | 56.25 ± 7.91 | 78.71 ± 6.95 | 93.10 ± 1.91 |
| 5% POVIDONE IODINE (GROUP II, n = 3) | ----- | -- | 3.29 ± 5.04 | 6.28 ± 9.80 | 52.39 ± 1.06 | 73.02 ± 4.14 | 89.12 ± 2.12 |
| SEABUCKTHORN (GROUP III, n = 3) | ----- | -- | -2.38 ± 4.78 | 2.74 ± 10.60 | 48.82 ± 4.66 | 81.03 ± 7.35 | 93.51 ± 2.13 |

In initial stages, in group I and III, histopathological inflammatory changes were comparable and relatively more severe characterized by abundant fibrinopurulent exudation with plenty of necrotic debris, neutrophils, degenerated cellular elements and clumps of bacterial masses. On day 7, marked fibrous tissue proliferation was evident in group II and III with lesser number of purulent foci compared to group I. The wound



D) Showing marked capillary proliferation (a) along with reduced surface necrotic area (b), 14 days following SBT ointment application on infectious cutaneous wounds in cow calves. E) Showing infiltration with macrophages and neutrophils (a) along with bundles of collagen fibers, although inflammatory reaction markedly reduced, 28 days following all the treatments. F) Showing granulation tissue covered by flat epithelium without rete pegs (a), 28 days following all the treatments.

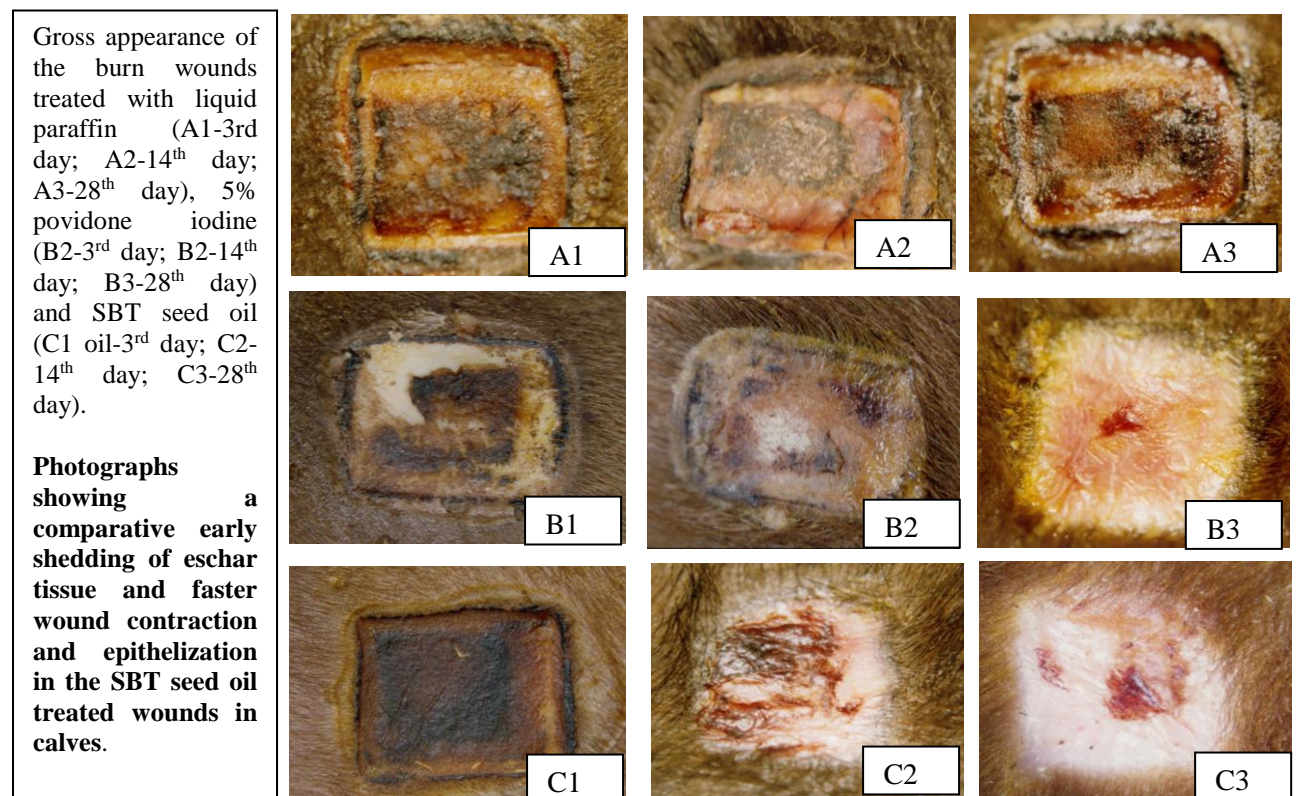
tissue was replaced by proliferating fibroblast and immature fibrous tissue but with incomplete epithelization and scattered neutrophilic infiltration by 14th day in group I whereas in group II, the inflammatory reaction was markedly reduced and majority of the wound gap was replaced by the mature fibrous tissue. The collagen fibers were aligned parallel to the skin surface and epithelization was almost complete. In group III also the wound was completely filled with mature fibrous tissue in the deeper parts with remnants of neutrophilic infiltration, but there was incomplete epithelization. In this group, the degree of inflammation, though markedly reduced, was more or less comparable to Group I. The extent of production of collagen was comparable to that of group II. At day 21st and 28th, the response to the healing was more or less similar in all the groups.

On the basis of above observations it is concluded that the wound healing of infected cutaneous wounds in cow calves was better following application of seabuckthorn ointment and 5% povidone-iodine in comparison to liquid paraffin.

7 Studies for the evaluation of wound-healing-promoting activities of Seabuckthorn on burn wounds

This study was conducted on 9 male calves divided into 3 equal groups viz I, II and III. Six equidimensional (3cmx3cm) full thickness cutaneous wounds, 3 on either side of the vertebral column were created aseptically under local infiltration anaesthesia on the dorsal thoracolumbar region of all the animals. The wounds were dressed once daily with, liquid paraffin, 5% Povidone-Iodine ointment and SBT seed oil in group I, II and III respectively. Dressing of the wounds was done once daily for 28 days. The methodology of wound healing evaluation remained more or less same as described in previous studies except that percent wound contraction studies and CST of wound swabs were not done.

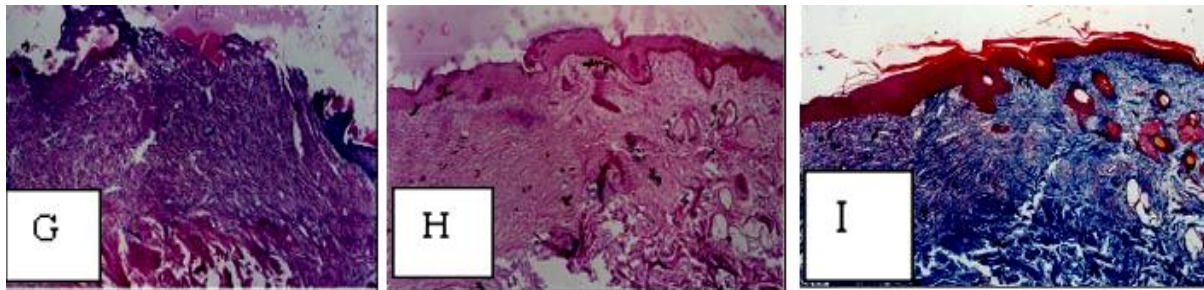
Results: The rectal temperature, respiration rate and heart rate remained within the normal range throughout the period of observations in all the groups. Similarly various haematological parameters like Hb, PCV, TEC, TLC and DLC remained within the normal range throughout the period of observations in all the groups. The clinical signs of inflammation around the wounds on day 3, varied from nil to mild intensity in group III and II and mild to moderate in group I. The signs of inflammation subsided by day 7th in group II and III, but up to day 11th in group I. Mild exudation was observed in two animals of group I on day 3rd; that increased slightly by 7th day and decreased gradually thereafter. The wound became dry after 7th day. No exudation was observed in any animal of group II and III.



The gross appearance of wounds on day 3 in group II and III was relatively more brownish to blackish and the zone of erythema was limited to just around the margins of the burn wounds. The appearance of burn wounds was pinkish with slight exudation in group I at this stage. On 7th day, slight detachment of surface layer of burn wounds was visible in all the animals of group III but no detachment of surface layer was observed in group I and II. The zone of inflammation was still limited to just wound margins in group II and III with no exudation. The wound exudation was visible in group I at this stage but disappeared by 11th day. The surface layer of all burn wounds of group III peeled out

along with dressing by day 14. The underlying healing tissue showed moderate contraction of wound margins with completely dry surface. Detachment of burn tissue in group II started by 14th day. The surface layer of burnt tissue fell in this group by 21st day revealing underlying surface of wound that was almost dry except at the centre. In most of animals of group I though the surface layer of burn tissue started slight detachment from underlying tissue but was still firmly adhered to be removed during dressing even on 28th day. The wound contraction in group III animals was considerably greater as compared to group II. No visible sign of infection was observed in any of the animals irrespective of treatment at any time during the experiment.

Histopathological examination of the wound on day 3 revealed severe inflammatory changes subsequent to burn and characterized by dermal coagulative necrosis with focal haemorrhagic and neutrophilic reactions along with desquamation of epidermis in all the animals. However in group III, the response to inflammation was comparatively mild as compared to other groups. The burn wounds on 7th



G) Showing young granulation tissue (a), 14th day after start of experiment in SBT group (H&E, 33X) in a burn wound.
 H) Showing junction of healing and burn wound, 21st day after start of experiment in SBT group (H&E, 33X).
 I) Showing junction of healing and burn wound, collagen fibres in blue colour and epidermal repair in red colour 21st day after start of experiment in SBT group (Masson's Trichrome, 33X).

day in all the groups were characterized by purulent inflammation, which was mild in group III and group II and moderate to severe in group I. Variable hydropic reaction and necrotic coagulative changes were seen in the epithelial tissue of epidermis and follicle, which were again minimal in group III. The fibroblastic proliferation was also noticed earlier in the wounds of group-III animals. At 14th day, relatively milder inflammatory reaction along with the deposition of mature collagen fibres in the healing tissue was indicative of faster healing in group-III animals. The epithelial regeneration in wounds was also faster in group-II and group-III animals as compared to group I. The epithelial hyperkeratinization was noticed in all the three groups with varying activity but leucocytic infiltration was still evident in the wounds of group-I animals when compared

to group-II and III animals. In group I the granulation tissue was still immature on 21st day. This indicated the relatively earlier burn-wound healing and remodeling in cases of Seabuckthorn oil treated wounds.

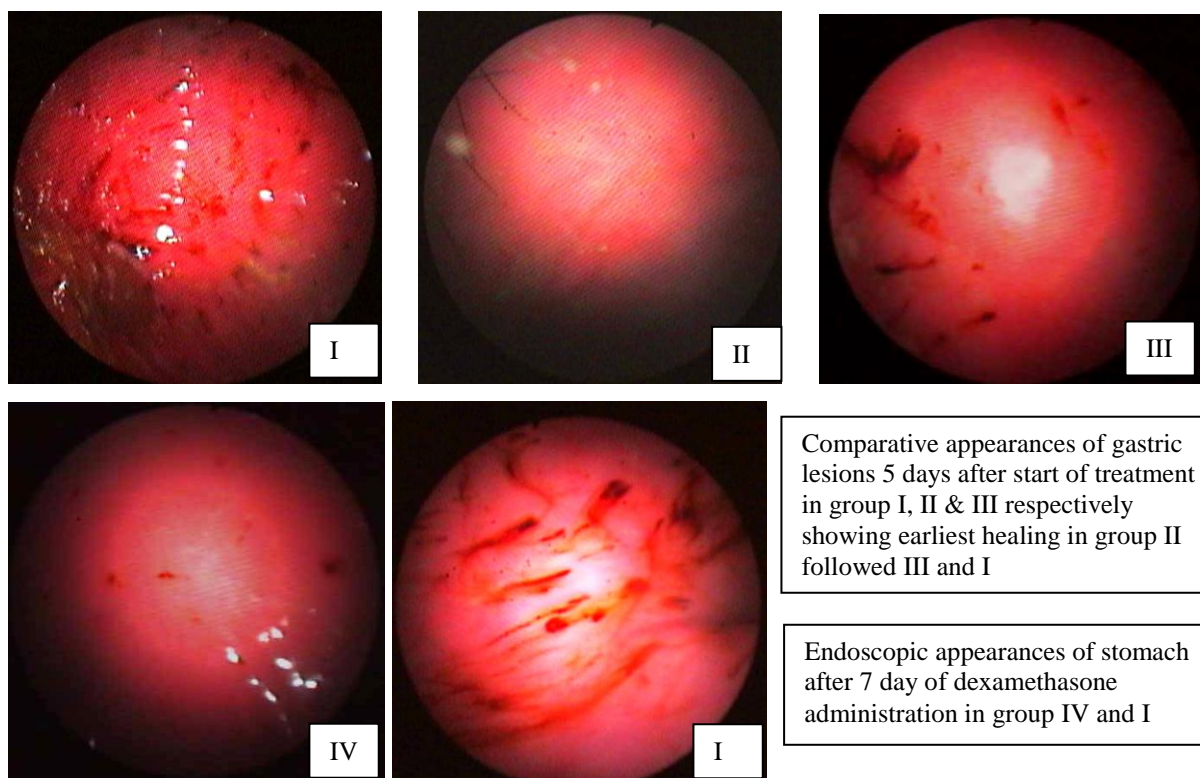
8 To study the efficacy of seabuckthorn on experimentally induced gastric ulcers in animals.

For this study, 27 adult healthy mongrel dogs were used. For development of gastric ulcer model, 15 animals were divided into 5 equal groups. Acetylsalicylic acid (Aspirin) @ 50 mg/kg orally b.i.d alone, Aspirin (same dose) plus Inj. Dexamethasone @ 1 mg/Kg I/M s.i.d., Inj Dexamethasone @ 1 mg/Kg I/M s.i.d., Inj Piroxicam @ 1mg/Kg I/M s.i.d and Inj. Piroxicam along with Inj. Dexamethasone both @ 1mg/kg I/M s.i.d were administered in group I, II, III, IV and V respectively for variable period. The development of gastric lesions and their subsequent healing progress were observed mainly by endoscopic examinations at regular intervals. Based on endoscopic observations, gastric lesions were categorized as sparse punctuate erosion, sparse linear and punctuate erosion, diffused linear and punctuate erosions and diffused erosion as grade 1, 2, 3 and 4 respectively. Animals were also subjected to regular clinical, haematological, plasma biochemical, radiological and histopathological examinations. Based on these observations, administration of Inj dexamethasone @ 1 mg/kg I/M s.i.d. for 14 days as used in Group III was found to be the most suitable and predictable model of gastric erosions/ulcerations leading to development of grade 4 gastric lesions. In this group, the gastric lesions started appearing 6th day onwards in all the animals and their intensity increased gradually. The haemoglobin and TEC values also started declining after day 10th in most of the animals. However, the clinical symptoms remained variable and did not follow any definite pattern despite endoscopic detection of even severe lesions. After discontinuation of dexamethasone on 14th day, spontaneous healing of these lesions as appreciated by endoscopy was found to occur within 15 days. The other groups did not result in to predictable gastric ulcer models. The group I animals took very long and with wide variable period (14-28 days) for development of gastric lesions. The other groups i.e II, IV and V resulted in to either severe lesions or with lack of repeatability in all the animals within a narrow time range. Therefore group III model was selected for subsequent studies.

To verify the therapeutic efficacies of seabuckthorn on gastric ulcers, 9 dogs were subjected to experimental creation of gastric ulcers as described above as in group III. The animals were randomly divided into three equal groups and the first group was kept

as negative untreated control; second group was treated with Omeprazole @ 10 mg/kg orally s.i.d. and the third group was administered with SBT seed oil @ 5ml/animal b.i.d. For verification of the prophylactic efficacy of seabuckthorn, another group (IV) of three animals was used. In these animals, SBT oil @ 5 ml/animal b.i.d. was administered simultaneously with ulcerogenic drug (Inj Dexamethasone for 14 days). The SBT oil was continued in the same dose in this group even after discontinuation of dexamethasone. The progress of ulcer healing was evaluated as described above. In group IV, the intensity of lesions was relatively less in the initial stages of gastric erosion/ulcerations development. But after 10th day the gastric lesion score was similar in all the groups. However, no animal of this group showed anorexia or any other ulcer related clinical symptoms like vomition, melena and weight loss as against all the animals of other groups throughout the period of study. After start of the treatment, the group II animals showed earliest endoscopically identifiable healing of gastric lesions followed by group IV, III and I respectively. In all the groups, gradual decline in haemoglobin and TEC values stopped after discontinuation of the ulcerogenic drugs and no significant variation was observed in between the groups.

Based on these observations it is concluded that oral feeding of seabuckthorn seed oil has prophylactic value in the development of gastric erosions/ulcerations and also has therapeutic potential to aid in their healing.



Comparative appearances of gastric lesions 5 days after start of treatment in group I, II & III respectively showing earliest healing in group II followed III and I

Endoscopic appearances of stomach after 7 day of dexamethasone administration in group IV and I

Publications: See annexure I

| Type of publication | No. |
|---|-----------|
| Research articles in referred journals | |
| 1. National | 5 |
| 2. International | 2 |
| Books and book chapters | 1 Chapter |
| Technical bulletins | - |
| Training manuals/instruction material including CDs | - |
| Popular articles | - |
| Leaflets developed for farmers | - |
| Coverage in press, TV, Radio | - |

Patents:

NIL

Technology developed: The use of seabuckthorn as a wound dressing agent and in management of gastric ulcers in animals was evaluated and was found efficacious.

The seabuckthorn may be commercially utilized by pharmaceutical and food industries for preparations of various wound dressing ointment and other medicinal/food products for prophylaxis against gastric ulceration. For this a few more studies for identification of key bioactive substances in the various plant components are required for standardization of such products before initiation of their commercial utilization. The study encompassing a greater data base is essential.

Infrastructure developed:

* **Major facility** of endoscopy in animals was developed for non-invasive diagnosis of internal conditions of oesophagus and stomach like gastric erosions/ulcers, tumours, foreign bodies and neoplasia which are otherwise remained undiagnosed or are diagnosed by more radical invasive/exploratory surgeries in veterinary practice.



Gastric endoscopy being performed in a dog

Equipments and major facilities

| Description | Number | Total Cost |
|--|---------------|-------------------|
| Equipments costing more than Rs 1 lakh (Endoscope with accessories) | 1 | Rs. 12,28,319 |
| Miscellaneous (Temperature and Moisture Control unit, computer and Trinacular Microscope with accessories etc) | 2, 1 | Rs. 2,83,201 |
| Major facility developed (Endoscopy) | | - |

Civil Works: NIL

Linkages developed:

Seabuck Cosmetic Division/Leh Berry Food division, Compact International limited, Greater Kailash , New Delhi: To achieve the objectives of the project timely, the said institute was approached. A linkage was developed with them for processing of seabuckthorn fruit/seeds/pulp for oil extractions at bigger scale by using their facilities.

Impact: The present study was the preliminary effort to verify the various wounds and ulcers healing capabilities of the Indian varieties of seabuckthorn. Seabuckthorn berry ointment and seed oil was found efficacious in treatment of aseptic incisional and excisional cutaneous wounds, burn wounds and for prevention of gastric ulcers in animals to some extent. In some cases the results were even better than routinely used medicines for such ailments. However, the role of seabuckthorn in treatment of subacute inflammation, infected wounds and gastric ulcers is yet to be properly established. Therefore, for recommending the use of seabuckthorn for preparation of certain drugs for above mentioned ailments, further studies are needed with the objectives of not only increasing the data size, but also to ascertain the more efficacious portion of the seabuckthorn. Additionally more studies are also needed to identify the bio-active substances which are responsible for such medicinal properties to this plant. Only then a comprehensive cost benefit analysis can be done for the eventual commercial utilizations of seabuckthorn by pharmaceuticals.

Human Resource Development (HRD)

Training organized: NIL

National Training of the project Team members NIL

Overseas training of the Project team Members NIL

| | |
|----------------------------------|------------|
| Technology Dissemination | NIL |
| Monitoring and Evaluation | NIL |
| Honours/Awards: | NIL |

How have process changes helped to bring about:

The Internet facilities as provided in the university through NATP was of immense values for retrieving the relevant information quickly and effectively round the clock. It saved a lot of time and resources leading to better progress of research work.

The liberal delegation of powers to respective PI's also cut through a lot of red tape and eased the procurement process of various items.

The adequate funding through NATP for purchase of costly equipments of endoscopy resulted in development of excellent diagnostic facilities at the institute which will be of great use in animal welfare.

More power to the PI's and Co PI's be delegated for future projects for more effective execution of work. These powers must include simplification of purchasing and auditing procedures.

The NATP must support those research projects for continuation of work in the future which have some positive outcome at this stage but requiring some more studies.

Utilization of results of the sub-project after termination of the sub-project:

As seabuckthorn was found efficacious in management of wounds and ulcers, it may be utilized in various medicines. However, more studies are needed to ascertain these properties. Research proposals in this regard are being prepared.

Financial: See annexure VII

Annexure I

1. Kumar Amit, Varshney A.C. and Tyagi S.P. 2004. Treatment of cutaneous burns with seabuckthorn oil in bovines: A clinical and haematological study. *Global seabuckthorn Research and Development*. (Accepted)
2. Kumar Amit, Varshney A.C. and Tyagi S.P. 2004. Evaluation of seabuckthorn (*Hippophae rhamnoides*) oil in the healing of cutaneous burns in bovines: A histological study. *Indian Journal of Animal Sciences*. (Accepted)
3. Munish Gupta, M. Singh, A.C. Varshney, S.P Tyagi and Amit Kumar. 2004. Efficacy of Seabuckthorn (*Hippophae rhamnoides*) in the healing of cutaneous wounds in dogs: Clinical and Haematological Studies. *Indian Journal of Veterinary Surgery*. (Accepted)
4. Amit Mahajan, S K Sharma, A C Varshney, S P Tyagi and Amit Kumar. 2004. Therapeutic efficacy of seabuckthorn (*Hippophae sp.*) in infected cutaneous wounds in bovines. *Indian Journal of Animal Sciences*. (Accepted)
5. Amit Mahajan, S. K. Shrama, R. K. Asrani, A.C. Varshney, S.P. Tyagi and Amit Kumar. 2004. Treatment of infected cutaneous wounds with seabuckthorn (*Hippophae sp.*) in bovines: A histopathological study. *Indian Journal of Veterinary Surgery*. (Accepted)
6. A. C. Varshney, S. P. Tyagi, Amit Kumar and V. Singh. 2004. Studies for the evaluation of anti-inflammatory activities of seabuckthorn (*Hippophae rhamnoides*) oil in canine. *Global seabuckthorn Research and Development*. (Accepted)
7. Manish Gupta, Mohinder Singh, R. K. Asrani, A.C. Varshney, S.P Tyagi and Amit Kumar. 2004. Evaluation of Seabuckthorn (*Hippophae rhamnoides*) ointment in the healing of cutaneous wounds in dogs: Histopathological and histochemical studies. *Indian Journal of Animal Sciences*. (Accepted)
8. A.C. Varshney and S.P. Tyagi. 2004. Seabuckthorn in cutaneous wound healing in animals. Seabuckthorn (*Hippophae L.*) A multipurpose wonder plant. Vol. 2. Ed. V. Singh, Indus Publishing Company, New Delhi

Annexure IV**Detailed list of Equipments:**

| Description | Number | Total Cost (in lac) |
|---------------------------------------|--------|---------------------|
| Endoscope with Accessories | 1 | 12.28319 |
| Temperature and Moisture Control unit | 2 | 1.07100 |
| Trinacular Microscope | 1 | 0. 51450 |
| Computer with accessories | 1 | 1.09049 |

Annexure VII**Financial outlay: Consolidated over all the centers**

Name of the Sub-project “Studies on the efficacy of Seabuckthorn (*Hippophae* sp.) on soft tissue repair with particular reference to cutaneous wounds, burns and gastric ulcers”

| S.No | Heads of Acctt. | Project Cost (original Sanction) | Total Amount Released | Actual Expenditure | | | | Remarks |
|-----------|--|----------------------------------|-----------------------|--------------------|-----------|-------------------------|----------|---------|
| | | | | 2002-2003 | 2003-2004 | 2004-2005 upto 31.12.04 | Total | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| A. | Recurring | - | - | - | - | - | - | - |
| 1 | Pay & Allowances | - | - | - | - | - | - | - |
| 2 | Contractual | 4.2966 | 3.1538 | 0.5104 | 1.4284 | 1.10919 | 3.04799 | |
| 3 | TA | 1.3 | 1.06642 | 0.4104 | .45602 | 0.2 | 1.06642 | |
| 4 | Operation exp. | 3.17 | 3.0686 | 0.95585 | 1.04275 | 1.07725 | 3.07785 | |
| 5 | Workshop/Training for farmers and other stakeholders | - | - | - | - | - | - | - |
| | Sub-Total | 8.7666 | 7.28882 | 1.87665 | 2.92717 | 2.38644 | 7.19026 | |
| B. | HRD | - | - | - | - | - | - | - |
| 1 | Training -Foreign -Domestic | - | - | - | - | - | - | - |
| 2 | Consultancy | - | - | - | - | - | - | - |
| | Sub-Total | - | - | - | - | - | - | - |
| C. | Non-recurring | - | - | - | - | - | - | - |
| 1 | Equipment | 15.11528 | 15.11528 | 11.09808 | 0.48720 | 3.52985 | 15.11513 | - |
| 2 | Civil Works | - | - | - | - | - | - | - |
| 3 | others | - | - | - | - | - | - | - |
| | Sub-Total | 15.11528 | 15.11528 | 11.09808 | 0.48720 | 3.52985 | 15.11513 | - |
| D. | Instt. charges | 0.7098 | 0.82401 | 0.3099 | 0.2999 | 0.21421 | 0.82401 | - |
| | Grand Total | 24.59168 | 23.22811 | 13.28463 | 3.71427 | 6.1305 | 23.1294 | - |

Financial (Consolidated) statement of Expenditure “Studies on the efficacy of Seabuckthorn (*Hippophae* sp.) on soft tissue repair with particular reference to cutaneous wounds, burns and gastric ulcers”

| Head | Total Sanction Allocation (Rs. In lakhs) (A) | Funds Received (Rs. In lakhs) | | | Total Funds Received (Rs. In lakhs) (B) | Difference between allocated funds & received (A-B) | Funds Utilized (Rs. In lakhs) | | | Total Funds Utilized (Rs. In lakhs) | Balances fund (Rs. In lakhs) as on Closing Date (A-C) | Unspent balance (Rs. In lakhs) as on Closing Date (B-C) |
|---------------------------------|--|-------------------------------|----------------|----------------|---|---|-------------------------------|----------------|----------------|-------------------------------------|---|---|
| | | 2002-03 | 2003-04 | 2004-05 | | | 2002-03 | 2003-04 | 2004-05 | | | |
| X. Recurring | | 2002-03 | 2003-04 | 2004-05 | | | 2002-03 | 2003-04 | 2004-05 | | | |
| TA | 1.3 | 0.6 | 0.3114 | 0.15502 | 1.06642 | 0.23358 | 0.4104 | 0.45602 | 0.2 | 1.06642 | - | - |
| Contratual Services | 4.2966 | 1.449 | 0.6868 | 1.018 | 3.1538 | 1.1428 | 0.5104 | 1.4284 | 1.10919 | 3.04799 | - | 0.10581 |
| Operational Expenses | 3.17 | 1.05 | 1.0495 | 0.9691 | 3.0686 | 0.1014 | 0.95585 | 1.04275 | 1.07725 | 3.07585 | - | (-) 0.00725 |
| Workshop | - | - | - | - | - | - | - | - | - | - | - | - |
| Total of A | 8.7666 | 3.099 | 2.0477 | 2.14212 | 7.28882 | 1.47778 | 1.87665 | 2.92717 | 2.38644 | 7.19026 | | 0.09856 |
| Y. Non-recurring | | | | | | | | | | | | |
| Equipment | 15.11528 | 10.7 | | 4.41528 | 15.11528 | - | 11.09808 | 0.4872 | 3.52985 | 15.11513 | | 0.00015 |
| Civil Works | - | - | - | - | - | - | - | - | - | - | - | - |
| Total Of Y | 15.11528 | 10.7 | | 4.41528 | 15.11528 | | 11.09808 | 0.4872 | 3.52985 | 15.11513 | - | 0.00015 |
| Z. Institutional Charges | | | | | | | | | | | | |
| Equipment | 0.7098 | 0.3099 | 0.2999 | 0.21421 | 0.82401 | (-) 0.11421 | 0.3099 | 0.2999 | 0.21421 | 0.82401 | - | - |
| Grand Total X+Y+Z | 24.59168 | 14.1089 | 2.3476 | 6.77161 | 23.22811 | 1.36357 | 13.28463 | 3.71427 | 6.1305 | 23.1294 | 0.09871 | .09871 |

Signature of PI

F&AO