Seabuckthorn (*Hippophae* sp.) for soft tissue repair in animals

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Seabuckthorn (*Hippophae* sp.) is one of the high potential medicinal plants that has been subjected to diverse kind of research. It grows naturally in high altitude areas as well as near sea shore in many countries of world like Russia, Britain, Germany, Finland, Romania, France, Central Asia, China, Nepal, Pakistan, India and Bhutan etc. In India, seabuckthorn grows in Himachal Pradesh, Jammu and Kashmir, Uttaranchal, Sikkim and Arunachal Pradesh. The seabuckthorn was reputed for its multifarious medicinal and nutritional values since ancient time and was used in Tibetan medicines as back as 8th century. However, this knowledge came to fore only in the later half of 20th century when the seabuckthorn plant was rediscovered by Xu Zhonglu of China in Tibetan plateau in 1952. Thereafter research on this plant led to discovery and verification of its multifarious medicinal properties. It was officially listed in Chinese pharmacopoeia in year 1977. This gave fillip to more extensive research on this plant. An international centre for research and training on seabuckthorn (ICRTS) was established at Beijing China in the year 1995 to promote international cooperation.

The seabuckthorn oil has versatile pharmacological activities which were attributed to its several bioactive substances. The fruits of seabuckthorn contained more than 190 kinds of bioactive substances and the oil contained 106 kinds of such substances. Out of these, there are 42 kinds of lipids and 36 kinds of flavonoids, 22 kinds of fatty acids 6 kinds of fat soluble vitamins and phenols (Zhemin, 1990). Seabuckthorn Fruits contains 18 amino acids (37-75 mg/g) including many essential ones in all its varieties (Zhang *et al*., 1990). Novruzov, (2001) reported that main antioxidant component of leaves are flavonoids, which comprises of leucoanthocyanidins, qercetin, sorhamnetin, epicatchin and flavonols. Chen *et al*. (1988) reported that the young leaves of seabuckthorn have high carotene and flavonoid contents. Singh (2001) reported that Major natural bio-active substances in seabuckthorn seed oil are flavone, vitamin E, vitamin C, carotenoids and unsaturated fatty acid (mainly linolenic and linoleic acids). Kallio *et al*. (2003) reported that seabuckthorn juice contain monosaccharides, glucose, fructose and malic and quinic acids. Singh *et al*. (2001) reported mineral composition of 8 populations of Indian seabuckthorn growing in Lahaul valley .The chromatographic analysis of the ripe fruit of seabuckthorn revealed the presence of malic acid, oxalic acid and other unidentified acid in it. These organic acids are known for certain physiological functions in the body such as reducing the toxic effects of some medicines like antibiotics, barbitals and preventing teratogenesis, damages from X-rays and side effects of oxygen therapy. The seabuckthorn contain significant contents of carotenoids (including β-carotene, β-4, 4, γ-carotene, biketone-β-carotene, zeaxanthin, lycopene and polyring-lycopene), flavoxanthin, progestin, cryptoxanthin, violaxanthin, neoxanthin and V_C, V_K, V_E (including α,β, γ-V_E), of which V_E and V_C are the major components of antioxidants (Qibikeva,1989). Steroids, flavonoids and vitamins (E and K) present in seabuckthorn are effective anticancerous and hepatoprotective agents.

For these reasons, it has great potential in the medical field, both as medicine and health food. The medical products made from it include simple prescriptions as well as complex ones, e.g. oil, soft extract, membranous preparations and aerosols. Seabuckthorn oil can be used to treat burns, skin radiations lesions, cervical erosions, gastric and duodenal ulcers etc. The different parts of Seabuckthorn have been used for the treatment of skin wounds and various ailments of cardiopulmonary and gastrointestinal system. 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. Olziikhutag (1968) 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. Guoli and Zhong (1989) recorded ulcer healing activities of seabuckthorn oil attributed to the presence of different flavonoids, terpenoids and some stress reducing compounds oil in seabuckthorn.. Zhang *et al.* (1989) treated coronary heart disease with TFH (Total flavonoids of Hippophae) and showed that TFH could remit angina and improves the mechanocardiography and ischaemic electrocardiogram. Zhang *et al.* (1989) found that both intraperitoneal injection of seabuckthorn oil and oral administration inhibited the tumour (sarcoma and lymphatic leukemia) in mice. Seabuckthorn plant has also been found to be effective in prevention and management of different age related problems particularly senile dementia. The drug significantly arrested the loss of acetylcholine level among the elderly people. Seabuckthorn oil has also been shown to possess antiviral and antibacterial activities. He reported the activity of Hiporamin (purified tannin fraction from the leaves of *Hippophae rhamnoides L)* against adenoviruses, Paramixoviruses, Herpes virus and Influenza virus. Antibacterial activity was reported against many strains of Staphylococcus aureus, Corynebacterium diphtheriae, E.coli, Proteus, Pseudomonas and Serratia etc. Its other useful pharmacological activities include anti-inflammatory capability (Varshney and Tyagi, 2004), cutaneous and burn wound healing ability (Varshney *et al.,* 2003), antigastroulcerative activity (Xiao *et al*.,1992), hepatoprotective (Cheng,1992), anti-cancerous (Li and Liu,1991), antilipemic and anti-arrhythmic (Fengming, 1989) properties.

Most of the research on seabuckthorn was however either done on laboratory animals such as guinea-pigs, rats and rabbits or directly on human patients. As far as domestic animals especially large and small domestic animals are concerned, not much work has been done. Therefore, we have undertaken a research project to verify the wound healing-supporting abilities of seabuckthorn in management of cutaneous incisional and excisional aseptic as well as infected wounds, burns and gastric ulcers in animals. In the study to evaluate the efficacy of seabuckthorn ointment on the healing of aseptic wounds in dogs by Gupta *et. al.* (2002), seabuckthorn ointment was compared with liquid paraffin and a commonly used antiseptic wound ointment 5% povidone-iodine. Grossly the signs of acute inflammatory reactions in cutaneous wounds were less pronounced and disappeared earlier both in seabuckthorn ointment and 5% povidone-iodine ointment treated animals. These signs subsided as early as on 3rd day as compared to around 7th day in paraffin treated wounds. Similarly the granulation tissue and scab formation in the wounds was noticed earlier in seabuckthorn and 5% povidone-iodine treated animals leading to early shedding of scab and achieving early complete

clinical healing within 14th –18th day as compared to liquid paraffin treated wounds where it took about 21-24 days. The seabuckthorn and 5% povidone-iodine ointment treated wounds also showed early and sustained greater wound contraction of 36% and 32% respectively on 7th day and 75% and 77% respectively on 14th day as compared to 17% and 69 % at 7th and 14th day respectively in liquid paraffin treated wounds. Grossly the wound healing process progressed almost comparably in seabuckthorn and 5% povidone-iodine ointment treated wounds. The histological observations of the healing tissue biopsies however, affirmed wound healing response in seabuckthorn ointment treated wounds even better than those treated by 5% povidone-iodine. It was evidenced by relative milder response of inflammation and greater fibroblastic proliferation in the wounds in the early stages of healing in seabuckthorn treated wounds. In later stages as well the process of epithelialization and the maturation and contraction of collagen fibers was found to be of most advanced nature in seabuckthorn treated animals. The collagen fibres were arranged parallel to the skin surface in cutaneous wounds in this group as compared to other two groups where the mature fibrous tissue appeared loosely arranged and comparatively disoriented even at 28th day of healing.

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. In another study conducted by Mahajan *et. al*. (2002) to evaluate the efficacy of seabuckthorn in the healing of infected cutaneous wounds in calves, the gross wound healing response was found to be comparable in seabuckthorn and 5% povidone-iodine ointment treated wounds. Whereas, histological observations of the healing tissue biopsies revealed better wound healing response in the initial stages in 5% povidone-iodine treated wounds. It was evidenced by relatively greater angiogenesis, fibroblastic proliferation and lesser fibrinopurulent exudation in 5% povidone-iodine treated wounds. However in later stages the wound healing response was comparable in seabuckthorn and 5% povidone-iodine ointment treated wounds which were nevertheless better than liquid paraffin treated wounds.

Similarly the seabuckthorn seed oil was compared with 5% povidone-iodine ointment and liquid paraffin in the healing of burn wounds in calves by Kumar, *et. al.* (2003) and Varshney, *et. al.*(2003). It was again observed that the signs of acute inflammatory reactions in wounds remained less pronounced in seabuckthorn oil and 5% povidone iodine treated animals as compared to paraffin treated animals. The healing progressed fastest in seabuckthorn oil treated wounds as evidenced by earliest shedding of burn eschar in this group. Histopathological examination of the healing tissue biopsies also substantiated the faster progression of healing process in the seabuckthorn oil treated

wounds. It was evidenced by presence of lesser number of neutrophils and mononuclear cells in the wound area, earlier and greater fibroblastic proliferation and its better organization towards the end of study period in seabuckthorn oil treated wounds.

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 melena 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.

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