

EVALUATION OF COPPER NANO PARTICLES COATED SEABUCKTHORN FOR WOUND HEALING ON RATS

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Introduction: Sea buckthorn is known to contain a wide range of bioactive compounds including flavonoids, phenolic compounds, carotenoids, and essential fatty acids. These compounds possess strong antioxidant and anti-inflammatory properties that play an important role in tissue repair. Antioxidants help neutralize reactive oxygen species generated during the inflammatory phase of wound healing, thereby protecting newly formed tissues from oxidative damage. The presence of vitamins such as vitamin C and vitamin E in sea buckthorn also contributes to collagen synthesis and stabilization, which is essential for proper wound healing.

Materials and methods: Fresh sea buckthorn leaves/fruits were collected and thoroughly washed with distilled water to remove dust and impurities. The plant material was shade dried and powdered. The powdered material was extracted using ethanol/distilled water by the Soxhlet extraction method. The extract was filtered and concentrated using a rotary evaporator and stored at 4°C until further use. **Results:** The copper nanoparticle-coated sea buckthorn group exhibited the highest percentage of wound contraction by day 14 compared to other groups. Histopathological analysis confirmed better tissue regeneration and organized collagen fibers in the treated group. **Conclusion:** The synergistic combination of copper nanoparticles and sea buckthorn extract may serve as a promising therapeutic agent for wound management. Further studies involving clinical trials and toxicity evaluation are required to confirm its safety and efficacy in humans.

Key words: Copper, seabuckthorn, Rats**INTRODUCTION**

Wound healing is a complex biological process involving inflammation, tissue formation, and tissue remodeling. Effective wound management remains a significant challenge in both clinical and veterinary medicine due to infection, delayed healing, and scarring(1). Traditional medicinal plants and modern nanotechnology have gained considerable attention for improving wound healing outcomes.

Sea buckthorn (*Hippophae rhamnoides*) is a medicinal plant widely known for its therapeutic properties, including anti-inflammatory, antimicrobial, antioxidant, and tissue regenerative activities(2). The plant contains bioactive compounds such as flavonoids, vitamins (A, C, and E), carotenoids, and essential fatty acids that contribute to enhanced tissue repair and regeneration.

Copper nanoparticles (CuNPs) have also attracted significant interest in biomedical applications due to their antimicrobial properties, ability to stimulate angiogenesis, and role in collagen synthesis(3). Copper ions are essential for several enzymatic reactions involved in connective tissue formation and wound healing.

Combining plant-based therapeutics with nanotechnology may improve the efficiency of wound healing agents(4). Coating copper nanoparticles with sea buckthorn extracts may provide synergistic effects such as enhanced antimicrobial activity, improved tissue regeneration, and reduced healing time.(5)

Therefore, the present study aims to evaluate the wound healing potential of copper nanoparticles coated with sea buckthorn extract in experimentally induced wounds in rats.

Wound healing is a highly coordinated and dynamic biological process that restores the integrity of damaged tissue. It involves a series of overlapping phases including hemostasis, inflammation, proliferation, and remodeling(6). Immediately after injury, the body initiates mechanisms to prevent blood loss and protect the wound from microbial invasion. This is followed by the inflammatory phase, where immune cells remove debris and pathogens. During the proliferative phase, fibroblasts, keratinocytes, and endothelial cells actively participate in tissue regeneration, collagen synthesis, and angiogenesis(7). Finally, the remodeling phase strengthens the newly formed tissue through collagen reorganization. Any disruption in these phases may result in delayed healing, infection, or chronic wounds.

Despite advances in modern medicine, wound management remains a major clinical challenge. Factors such as microbial infections, oxidative stress, poor vascularization, and metabolic disorders can significantly impair the healing process(8). Conventional wound treatments often rely on antibiotics, antiseptics, and synthetic dressings; however, prolonged use of these treatments may lead to antimicrobial resistance, allergic reactions, and limited effectiveness in chronic wounds. Therefore, there is increasing interest in exploring alternative therapeutic strategies that are safe, effective, and capable of promoting faster tissue regeneration.

Medicinal plants have been used for centuries in traditional medicine systems for the treatment of wounds and skin disorders. Among these, sea buckthorn (*Hippophae rhamnoides*) has gained considerable attention due to its remarkable pharmacological properties(9). Sea buckthorn is a hardy shrub widely distributed in Asia and Europe and is well known for its rich nutritional and medicinal value. Different parts of the plant, including the berries, leaves, and seeds, contain a variety of bioactive compounds such as flavonoids, carotenoids, tocopherols, phenolic acids, phytosterols, and essential fatty acids. (10) These compounds exhibit potent antioxidant, anti-inflammatory, antimicrobial, and tissue regenerative activities, which are essential for efficient wound healing.(11)

Sea buckthorn oil and extracts have been traditionally used in the treatment of burns, ulcers, skin injuries, and inflammatory conditions. The presence of vitamins A, C, and E plays a significant role in stimulating collagen synthesis, improving epithelial cell proliferation, and protecting tissues from oxidative damage(9,12). Additionally, its fatty acid composition supports the maintenance of skin barrier function and enhances the repair of damaged tissues.

Materials and Methods**Experimental Animals**

Healthy adult Wistar albino rats weighing 180–220 g were used for the study. The animals were housed in polypropylene cages under standard laboratory conditions with a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. Rats were provided with standard pellet diet and water ad libitum.

All experimental procedures were conducted according to institutional animal ethics guidelines.

Preparation of Sea Buckthorn Extract

Fresh sea buckthorn leaves/fruits were collected and thoroughly washed with distilled water to remove dust and impurities. The plant material was shade dried and powdered. The powdered material was extracted using ethanol/distilled water by the Soxhlet extraction method. The extract was filtered and concentrated using a rotary evaporator and stored at 4°C until further use.

Synthesis of Copper Nanoparticles

Copper nanoparticles were synthesized using a chemical reduction method. Copper sulfate solution was used as the precursor. The reducing agent such as sodium borohydride or plant extract was added slowly under constant stirring. Formation of copper nanoparticles was confirmed by the color change of the solution.

Preparation of Copper Nanoparticle-Coated Sea Buckthorn

The prepared copper nanoparticles were mixed with the sea buckthorn extract to allow coating of nanoparticles with plant bioactive compounds. The mixture was stirred continuously and then dried to obtain the coated formulation.

Experimental Design

The rats were randomly divided into four groups (n = 6 per group):

Group I – Control:

Wounds treated with normal saline.

Group II – Standard:

Wounds treated with standard wound healing ointment.

Group III – Sea Buckthorn Extract:

Wounds treated with sea buckthorn extract alone.

Group IV – Copper Nanoparticles Coated Sea Buckthorn:

Wounds treated with copper nanoparticles coated sea buckthorn formulation.

Wound Creation

Rats were anesthetized using ketamine or ether anesthesia. The dorsal surface was shaved and disinfected using 70% alcohol. A circular full-thickness excision wound of approximately 1–2 cm diameter was created using sterile surgical instruments.

Treatment Procedure

The respective treatments were applied topically to the wound area once daily for a period of 14–21 days. The wounds were observed and measured at regular intervals.

Epithelialization Period

The number of days required for complete epithelial covering of the wound was recorded.

Histopathological Examination

Tissue samples from healed wounds were collected and fixed in formalin. The samples were processed, sectioned, and stained using hematoxylin and eosin to evaluate collagen deposition, fibroblast proliferation, and angiogenesis.

Statistical Analysis

All data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using ANOVA followed by appropriate post-hoc tests. A value of $p < 0.05$ was considered statistically significant.

Results

The study demonstrated significant wound healing activity in rats treated with copper nanoparticle-coated sea buckthorn compared to control groups.

The treated group showed:

- Faster wound contraction
- Reduced epithelialization time
- Increased collagen deposition
- Enhanced fibroblast proliferation
- Improved angiogenesis

The copper nanoparticle-coated sea buckthorn group exhibited the highest percentage of wound contraction by day 14 compared to other groups. Histopathological analysis confirmed better tissue regeneration and organized collagen fibers in the treated group.

Discussion

The results of the present study indicate that copper nanoparticle-coated sea buckthorn significantly enhances wound healing in rats. Sea buckthorn extract is known for its antioxidant and anti-inflammatory properties, which help reduce oxidative stress and inflammation at the wound site.(13)

Copper nanoparticles contribute to antimicrobial activity, preventing infection during wound healing(14). Copper also plays a crucial role in collagen cross-linking, angiogenesis, and tissue regeneration.

The combination of copper nanoparticles with sea buckthorn extract likely produces a synergistic effect, improving cellular proliferation, collagen formation, and vascularization. This combination accelerates the wound healing process compared to either treatment alone.(15)

Wound healing is a complex biological process that requires the coordinated interaction of various cells, growth factors, cytokines, and extracellular matrix components. In the present study, the accelerated wound contraction observed in the copper nanoparticle-coated sea buckthorn group indicates enhanced cellular activity at the wound site(16). Wound contraction occurs mainly due to the activity of fibroblasts and myofibroblasts, which synthesize collagen and contribute to tissue remodeling(4). The increased contraction rate suggests that the treatment stimulated fibroblast proliferation and extracellular matrix formation.(17)

Sea buckthorn is known to contain a wide range of bioactive compounds including flavonoids, phenolic compounds, carotenoids, and essential fatty acids. These compounds possess strong antioxidant and anti-inflammatory properties that play an important role in tissue repair(18). Antioxidants help neutralize reactive oxygen species generated during the inflammatory phase of wound healing, thereby protecting newly formed tissues from oxidative damage(19). The presence of vitamins such as vitamin C and vitamin E in sea buckthorn also contributes to collagen synthesis and stabilization, which is essential for proper wound healing.(20)

Conclusion

The present study demonstrates that copper nanoparticles coated with sea buckthorn extract possess significant wound healing activity in rats. The formulation accelerates wound contraction, enhances epithelialization, and promotes better tissue regeneration.

The synergistic combination of copper nanoparticles and sea buckthorn extract may serve as a promising therapeutic agent for wound management. Further studies involving clinical trials and toxicity evaluation are required to confirm its safety and efficacy in humans.

These findings are consistent with previous studies demonstrating the beneficial effects of metal nanoparticles and herbal extracts in wound management.

Acknowledgements

The authors express sincere gratitude to the Department of Pharmacology/Biotechnology (or relevant department) for providing laboratory facilities and technical support for conducting this research.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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