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Effect of Neem Extract with Zinc Oxide Nanoparticles for Burn Wound Treatment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to determine the clinical efficiency of neem extract with Zinc Oxide (ZnO) nanoparticles on burn wound healing treatment in Rabbit model. Sixteen (16) male New Zealand white rabbits were assigned into four groups, each with four rabbits, and were kept together for 28 days. Group T_0 as the control; T_1 represented burn healing using neem extract; T_2 represented burn healing using neem extract with ZnO nanoparticles; and T_3 represented burn healing using a commercial drug (Bactrocin®). Data were accumulated on the healing rate, healing time, and WBC count. Healing rate was calculated at weekly interval and the healing time was observed regularly

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and calculated from day 7 at weekly interval and WBC count were investigated using an auto analyzer. The result showed that the healing rate (%) were T_2 group= 93.75±1.25, T_3 group=86.75±0.479, T_1 group=84.25±1.75, and T_0 group=70.75±2.25. Among this result the healing rate increased significantly in T_2 (93.75±1.25) group, better by T_3 (86.75±0.479), good by T_1 (84.25±1.75) and worst by T_0 (70.75±2.25) group. The application of neem extract with ZnO nanoparticles have also showed the quickest healing time (24.5±0.64 day) significantly ($P<0.01$). At weekly interval WBC count (cells/µl) was varied significantly, the highest value was discovered in the T_2 group. The study's overall findings demonstrated that using neem extract with ZnO nanoparticles resulted in faster wound healing with a shorter time requirement. It indicates that wound healing without the use of antibiotics was possible and using neem extract can be advised for the treatment of burn wounds.

Keywords: Neem; ZnO nanoparticles; commercial drug; wound; rabbit.

1. INTRODUCTION

Wound is defined as any anatomical and physiological disruption in skin structure leading to skin cell damage [1]. Thereby, wound healing is a complex and overlapping process of reaction and interaction among cells and mediators to return natural skin ability, beginning immediately after skin loss [2]. Immediately after injury in the first stage of homeostasis, vascular constriction and clot formation caused by fibrin and platelets stop the bleeding [3]. Platelets release growth factors and attract neutrophil macrophage, and other inflammatory cells into the wound site. These cells kill microbes, break wound debris, secrete cytokines and produce reactive oxygen species [4].

Neem (*Azadirachta indica*) ingredients are applied in Ayurveda, Unani, Homeopathy, and modern medicine for the treatment of many infectious, metabolic, or cancer diseases [5]. Different types of preparations based on plants or their constituents are very popular in many countries in disease management. In this vista, Neem, a member of the Meliaceae family, originated in the Indian sub-continent and its distribution has spread worldwide commonly found in India, Pakistan, Bangladesh, and Nepal, it has therapeutic implications in disease cure and formulation based on the fact that neem is also used to treat various diseases. The United Nations acknowledged the importance of this tree and entitled it the "Tree of the 21st century." The extraction solubilizes secondary metabolites in the form of several such polyphenols as tannins, phenols, terpenoids, flavonoids, saponins, and steroids that have complex structures involving many chiral centers that determine their biological activity [6,7]. It has a complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids, and such types of ingredients play a role in diseases' management through modulation of various genetic pathways and other activities. Nimbidin, as a major constituent extracted from neem seeds, demonstrated several biological activities, such as anti-inflammatory, anti-pyretic, anti-diabetic, anti-fungal, and anti-ulcer activities. Nimbolide has been shown to exert anti-malaria and antibacterial effects [8].

Nanotechnology is a rapidly growing and challenging research area that has vexed scientists' interest in a variety of biomedical applications [9]. Nanoparticles have been introduced into various aspects of life, aspects such as industry, health, food, biomedical and cosmetics industries. A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometres (nm) in diameter. Zinc-containing preparations have been shown to improve wound healing and control bacterial infection at the tissue site. They also aided in the formation of granulation tissue at the site of the wound [10]. Zinc Oxide is an inorganic material that exhibits unique properties including semiconductor, a wide range of radiation absorption, piezoelectric, pyroelectric and possesses high catalytic activity. Zinc oxide nanoparticles (ZnO-NPs) are the most interesting of the inorganic metal oxides because they are inexpensive to manufacture, clean, and simple to prepare [11]. Zinc oxide nanoparticles are one of the most commonly used metal oxide nanoparticles for biomedical purposes such as wound healing, antimicrobial, and antiinflammatory properties [12]. These studies have yielded promising outcomes. Zinc oxide nanoparticles have very unique antibacterial properties, preventing wound infection by inhibiting bacterial colonies of *Staphylococcus aureus* and *Escherichia coli* in wounds. Furthermore, zinc oxide nanoparticles promote wound healing [13].Very few research works has

been done to know the effect of the neem extract with Zinc oxide nanoparticles on wound healing treatment. So, considering the above properties and the therapeutic use of neem extract, ZnO nanoparticles, this experiment was carried out for burn wound treatment using rabbit model to know the clinical efficacy of neem with ZnO-NPs on burn wound healing activity.

2. MATERIALS AND METHODS

2.1 Experimental Period and Location

The experiment was conducted during the period from 05 June to 17 July, 2022 at animal research shed of the department of Physiology and Pharmacology, HSTU, Dinajpur.

2.2 Collection of Rabbit

Around nearly 2.5 months age 16 male rabbits (New Zealand White) were collected at Rangpur.

2.3 Experimental Treatments

Sixteen male rabbits of near about 2.5 months were chosen and divided into four groups i.e. T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs and T_3= Hot iron burned and treated with commercial drug (Bactrocin®).

2.4 Collection and Preparation of Extract

The HSTU campus is where the neem leaves were collected. The sample preparation was followed by [14].

2.5 Combined used of Neem Extract and ZnO-NPs

The preparation was made by combining 4g of neem extract and 1g of ZnO-NPs (Brand: Adnano Technologies Private Limited). In the hot iron burn wound locations; this mixer was then employed twice a day in each group. This is a new combination of the studied materials that has been used for the first time in the research based on the rabbit model [15].

2.6 Data Collection

2.6.1 Recording of wound healing time

Any formation of the scare was also noted carefully as the sign of healing at the time interval of 7 days for each treatment group.

2.6.2 Determination of healing rate

The healing rate was determined from each treatment group at an interval of 7 days. It was calculated by the greatest average wound margin distance from the wound centre divided by the time to complete wound closure is proposed. Healing rate was calculated at weekly interval by applying the formula:

> Wound Closure (%) = [(Area of the wound on day 0 – area of the wound on indicated day)/Area of the wound on day 0]*100 [16].

2.6.3 Determination of healing time

The healing time of rabbit was obtained from the day of wound creation to final day count of the research. A weekly interval was used to calculate the healing time for each treatment group.

2.6.4 Determination of WBC enumeration

WBC count were carried out by Automated Mindray BC-10 Haematology Analyzer and done manually at Green diagnostic center, Dinajpur. The WBC was count for each treatment group every week.

2.7 Statistical Analysis

The results of various biochemical parameters were done using SPSS version 22 and Microsoft Excel. Statistically significant differences between group means were determined by analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Wound Healing Rate (%)

In Table 1 the result represent the healing rate of the burn wounded rabbits gradually increased from 1st week to 4th week of the study period and showed significant variation among the experimental treatments ($P<0.01$). The highest healing rate (%) was obtained from T_2 (Neem extract and ZnO-NPs) treated group (93.75±1.25) followed by T_3 (Bactrocin®) treated group (86.75±0.479), T_1 (Neem extract) (84.25±1.75) and T_0 (Control) group (70.75±2.25). Amongst the studied treatments the T_2 (Neem extract with ZnO-NPs) treated group showed the best performance in relation to

healing rate throughout entire the study period (1st week to 4th week). The lowest result was obtained from non-treated group. Neem promotes wound healing activity through increased inflammatory response and neovascularization in animals. Previous investigations have shown that the well-known antibacterial compound ZnO is effective at very low bacterial concentrations. Zinc oxide nanomaterial has been known as an antibacterial agent [17]. This is in accordance with results obtained by Gunalan et al. [12] which demonstrated that the biosynthesized against bacteria.

3.2 Healing Time

The recovery times (in days) needed for each treatment during the study period was displayed in Table 2. The study's findings demonstrated a statistically significant difference between the experimental treatments (P<0.01). The T_2 (Neem extract with ZnO-NPs) treatments performed best (24.5±0.6455 days) which takes minimum days than other treatments to heal the artificially burn wound of rabbit followed by T_3 (Bactrocin®) treatments (26.5±1.04 days) and T_1 (Neem extract) treatment (28.25±0.629 days). In contrast, the control group needs the most time to heal the artificial burn wounds (33.75±0.75 days). The glycolysis process, which includes converting carbohydrates into energy, may require repeating redox reactions, which is one potential method of interaction between Zn ions and plant phytochemicals. These interactions result in the fastest and highest healing of the rabbit's burn wound [18]. Nanoparticles had been chosen to be involved in this study from the point of their rapidly growing concepts in tissue engineering for bone regeneration as mentioned by Mirzaei and Darroudi [19]. Zinc oxide nanoparticles were selected because of their low toxicity, low cost and its availability compared to the other different natural non-organic metallic NPs, and Zn^{2+} ions are well-known to stimulate bone formation and mineralization and to have an active role in the proliferation of osteoblastic cells. It indicates that the ZnO-NPs improve the healing of burn wounds faster than others.

Table 1. Effects of neem extract, neem extract with ZnO-NPs and Bactrocin® on hot iron burn wound healing rate (%) of rabbit weekly

Treatment	1st Week	2nd Week	3rd Week	4th Week
T_0	21.75±1.136[a]	35±1.871[a]	52.25±1.652[a]	70.75±2.25[a]
T_1	25±1.875[a]	45.5±3.279[b]	67.75±2.287[b]	84.25±1.75[b]
T_2	44.75±1.315[c]	64.5±1.443[c]	83.25±1.25[c]	93.75±1.25[c]
T_3	36.75±1.031[b]	59.75±1.931[c]	74±1.915[b]	86.75±0.479[b]
P-Value	0.000***	0.000***	0.000***	0.000***

**** Significant at 1% level, Mean for wound recover size with different superscript within the rows were significantly different at p<0.01.*

Here, T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

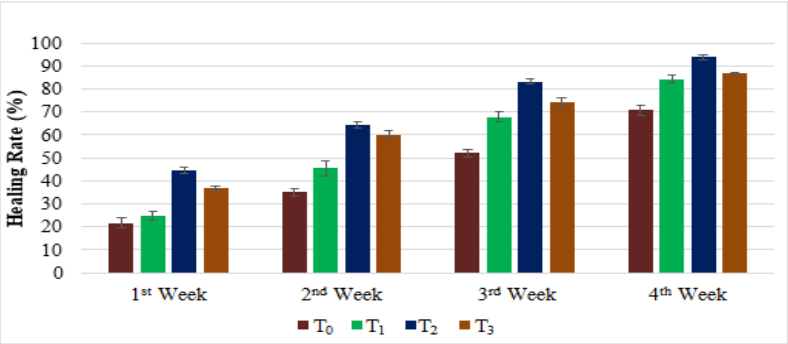

Fig. 1. Effects of neem extract, neem extract with ZnO-NPs and Bactrocin® on hot iron burn wound healing rate (%) of rabbit

Here, T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

Table 2. Effects of neem extract, neem extract with ZnO-NPs and (Bactrocin®) on healing time (days) of rabbit at 4th week

Treatment	Healing Time (Days)
T_0	33.75 ± 0.75^{c}
T_1	28.25 ± 0.629^{b}
T_2	24.5 ± 0.6455^{a}
T_3	26.5 ± 1.04^{ab}
P-Value	0.000***

**** Significant at 1% level, Mean for wound recover size with different superscript within the rows were significantly different at p<0.01*

Here, T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

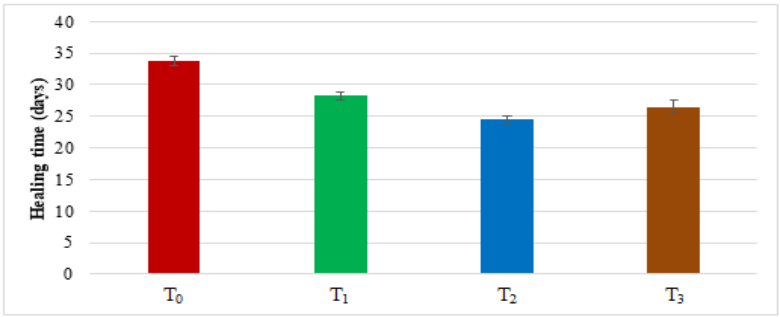

Fig. 2. Effects of neem extract, neem extract with ZnO-NPs and Bactrocin® on hot iron burn wound healing time (days) of rabbit at 4th week

Here, T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

Table 3. Effects of neem extract, neem extract with ZnO-NPs and Bactrocin® on WBC count (cells/µl) of rabbit at weekly interval

Treatment	Day 0	1st week	2nd week	3rd week	4th week
T_0	6000 ± 35.82^{c}	8500 ± 41.82^{a}	7800 ± 38.82^{b}	7200 ± 37.82^{c}	5500 ± 34.82^{b}
T_1	4500 ± 40.8^{a}	8900 ± 36.82^{b}	8000 ± 40.82^{c}	6300 ± 35.82^{b}	5075 ± 39.824^{a}
T_2	4500 ± 38.8^{a}	9000 ± 39.82^{c}	8600 ± 35.82^{d}	7200 ± 39.82^{c}	5800±42.82
T_3	5200 ± 32.82^{b}	8800 ± 36.87^{b}	7000 ± 33.82^{a}	6000 ± 42.82^{a}	5700±38.82
P-Value	0.000***	0.000***	0.000***	0.000***	0.000***

**** Significant at 1% level, Mean for wound recover size with different superscript within the rows were significantly different at p<0.01*

Here, T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

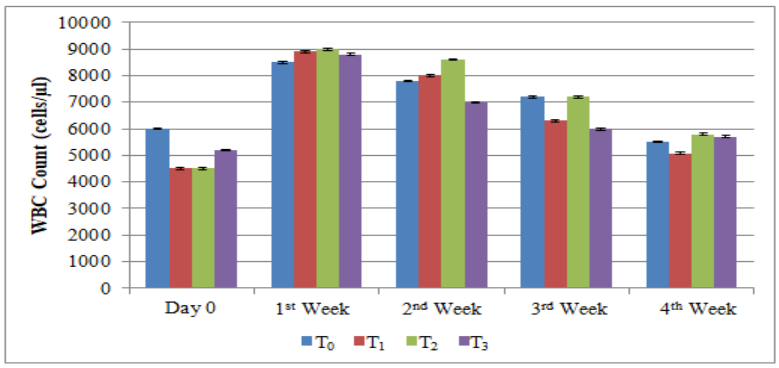

Fig. 3. Effects of neem extract, neem extract with ZnO-NPs and Bactrocin® on WBC count (cells/µl) of rabbit

Here, T_0= Control/non-treated group, T_1= Hot iron burned and treated with neem extract, T_2= Hot iron burned and treated with neem extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

3.3 WBC Count

Table 3 shows that the WBC count of the different studied treatments influenced significantly by the different experimented groups (P<0.01). The present study revealed that the WBC count increased greatly in the non-treated group/control group (at day 0 and 3rd week) which was 6000±35.82 and 7200±37.82 cells/µl respectively. At day 1st, 2nd, 3rd and 4th week, the maximum WBC count was counted on T_2 (Neem extract with ZnO-NPs) treated group (9000±39.82, 8600±35.82, 7200±37.82 and 5800±42.82 respectively). The lowest WBC count was obtained from T_3 (Bactrocin®) treated groups in most of the cases. The crucial role of WBC in defending the body against infection and tissue damage is well known. This supports previous reports that some commonly prescribed medicinal plants contains agents that stimulate the production of leucocytes [20,21]. This suggests that the extract may have immune boosting effect on the animals. Increased vascular permeability may possibly be the cause of such consequences. Mistletoe extract administration appears to have a stimulating effect on the immune system's effector cells. Immune enhancers are typically advised to strengthen and balance aging bodily systems and help the immune system combat foreign invaders like bacteria and viruses [22].

4. CONCLUSIONS

The results of the study showed that applying neem extract and ZnO nanoparticles to burn wounds in rabbits resulted in quicker wound healing with a reduced minimum day's required. This suggests that hot iron wound healing is still achievable without applying any commercial drug like Bactrocin®.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. George Broughton II, Janis JE, Attinger CE. The basic science of wound healing. Plastic and Reconstructive Surgery. 2006;117(7S):12S-34S.
2. ŠITUM M, Kolić M. Chronic wounds: differential diagnosis. Acta medica Croatica: Časopis Akademije medicinskih znanosti Hrvatske. 2013;67(Suplement 1):11-9.
3. Reinke JM, Sorg H. Wound repair and regeneration. European Surgical Research. 2012;49(1):35-43.
4. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen. 2008;16(5):585-601.
5. Brahmachari G. Neem—an omnipotent plant: A retrospection. Chembiochem. 2004;5(4):408-21.
6. Oluwafemi RA, Oluwayinka EO, Alagbe JO. Effect of dietary supplementation of neem oil (*Azadirachta indica*) on the growth performance and nutrient digestibility of weaned rabbits. Journal of Science, Computing and Engineering Research. 2020;1(4):100-5.
7. Alagbe JO, Betty AM. Heamatologfical and serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* bark mixture. International Journal of Biological, Physical and Chemical Studies. 2019; 1(1):08-15.
8. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Current science. 2002;1336-45.
9. Akbar S, Tauseef I, Subhan F, Sultana N, Khan I, Ahmed U, Haleem KS. An overview of the plant-mediated synthesis of zinc oxide nanoparticles and their antimicrobial potential. Inorganic and Nano-Metal Chemistry. 2020;50(4):257-71.
10. Rostan EF, DeBuys HV, Madey DL, Pinnell SR. Evidence supporting zinc as an important antioxidant for skin. International Journal of Dermatology. 2002;41(9):606-11.
11. Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, Gaurav K, Karthik L, Rao KB. Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012;90:78-84.
12. Gunalan S, Sivaraj R, Rajendran V. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International. 2012;22(6):693-700.
13. Rao GV, Selvaraj J, Ramanan RS, Radhakrishnan N, Manohar BM. Efficacy

of some indigenous medicines in wound healing in rats. Indian journal of animal sciences (India); 2003.

14. Mehedi M. Wound healing activity of nanoparticles with traditional plants in chemically burned rabbit model. M.S. Thesis. Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur; 2021.
15. Ali K, Dwivedi S, Azam A, Saquib Q, Al-Said MS, Alkhedhairy AA, Musarrat J. Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. Journal of Colloid and Interface Science. 2016;472:145-56.
16. Sato H, Ebisawa K, Takanari K, Yagi S, Toriyama K, Yamawaki-Ogata A, Kamei Y. Skin-derived precursor cells promote wound healing in diabetic mice. Annals of Plastic Surgery. 2015;74(1):114-20.
17. Xu Y, Wang W, Ma L, Cui X, Lynch I, Wu G. Acute toxicity of Zinc Oxide nanoparticles to silkworm (*Bombyx mori* L.). Chemosphere. 2020;259:127481.
18. Tewari-Singh N, Goswami DG, Kant R, Ammar DA, Kumar D, Enzenauer RW, Casillas RP, Croutch CR, Petrash JM, Agarwal R. Histopathological and molecular changes in the rabbit cornea from arsenical vesicant lewisite exposure. Toxicological Sciences. 2017;160(2):420-8.
19. Mirzaei H, Darroudi M. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics International. 2017;43(1):907-14.
20. Al-Mamary MA. Antioxidant activity of commonly consumed vegetables in Yemen. Mal J Nutr. 2002;8(2):179-89.
21. Imoru JO, Eno AE, Unoh FB, Nkanu EE, Ofem OE, Ibu JO. Haematopoietic agent (s) in the crude extract from the leaves of Viscum album (Mistletoe). Nigerian Journal of Health and Biomedical Sciences. 2005;4(2):139-45.
22. Bendich A. Physiological role of antioxidants in the immune system. Journal of Dairy Science. 1993;76(9):2789-94.

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