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Biosynthesis of Aloe Vera Gel with Zinc Oxide Nanoparticles (Zno-Nps) for Burn Wound Treatment in Rabbit Model

Chandan Barman [a*], Rakibul Islam [a], Md. Mahmudul Hasan [a], Misrat Masuma Parvez [a], Md. Rashid Anjum [a] and Mst. Nasrin Banu [b]

[a] Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Bangladesh.
[b] Faculty of Veterinary and Animal Science, Gono Bishwabidyalay, Dhaka, Bangladesh.

Authors' contributions

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

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ABSTRACT

To see the efficacy of combined preparation of aloe vera gel and Zinc Oxide nanoparticles (ZnO NPs) on burn wound treatment has been studied in Rabbit model. Sixteen male New Zealand White male rabbits were divided into four different groups and data were collected on healing rate, healing time and WBC count in artificial hot iron burned rabbit model. Group T_0, T_1, T_2 and T_3 were as control, aloe vera gel, aloe vera gel with ZnO nanoparticles and Bactrocin® respectively. Area of the wound and healing time was determined at weekly interval. WBC was enumerated weekly interval using a hematology analyzer. The experimental results showed that rate was significantly increase in T_2 (94.75±1.31) group, better by T_3 treated group (92.50±1.32), good by T_1 (85.50±2.21) and worst by T_0 (70.50±2.72). The White Blood Count (WBC) count as greatly increased in the non-treated group in most of the cases (Day 0 and Day 28), but the best WBC count was found in

**Corresponding author: Email: chandanroy80.cr@gmail.com;*

(Bactrocin®) in the rest of the cases (9300±37.50, 8500±37.36 and 6600±24.25 cells/µl for 1st week, 2nd week and 3rd week respectively), while the good count was obtained from T_2 (Aloe vera gel with ZnO nanoparticles) treatment. The overall result of the study showed that the use of aloe vera gel with ZnO nanoparticles has given faster wound healing with minimum days requirement, which proved that without antibiotics, wound healing was also possible by using of biosynthetic ZnO nanoparticles and can be recommended for burn wounds healing treatment in rabbits.

Keywords: Aloe vera; ZnO nanoparticles; commercial drug; wound; rabbit.

1. INTRODUCTION

An injury to the skin or other organic tissue known as a "wound" is one that is primarily brought on by heat, radiation, radioactivity, electricity, friction, or contact with chemicals. The most serious and physically crippling, burns affect practically every organ system and have a high death and morbidity rate. Burns in small animals affect trauma patients who develop significant skin sores that eventually spread to the subcutaneous and musculature layers, causing a host of organic and metabolic problems. Burn injuries result in significant mechanical damage to the cells of each integument layer. An animal's skin and flesh are particularly vulnerable to wounds, which are trauma-induced injuries. Burns can cause minor medical issues or serious emergencies.

Over the years, a variety of medicinal herbs have also been utilized for the care and healing of wounds [1]. Several studies on aloe vera have been conducted and shown to be effective in the prevention and healing of skin wounds. Historically, it has been used as a traditional medicine for the treatment of skin problems, infections, and other ailments in India, as well as as an anti-fungal, anti-diabetic, and anti-hypertensive agent in Chinese, Mexican, and Trinardiancultures [2]. Furthermore, it is listed in the Chinese and American Pharmacopeas, as well as the Thai herbal fundamental public health drug list [3]. In ancient Egypt, this plant was considered as the "plant of immortality" and was included in the traditional healthcare systems of various cultures, including the Arabs, the Egyptians, Greeks, Romans, Indians, Japanese, Koreans, and Chinese, to treat a variety of skin disorders such as burns and wounds, psoriasis, and dermatitis [4,5]. Since ancient periods, aloe vera has been used ethno pharmacologically to treat the healing of burns and wounds [6], which has been confirmed further via multiple studies by using its leaves and latex, fresh gel, juice, cream, solution, or manufactured solutions, etc.[7,8]. Several in-vitro and in-vivo studies, as well as clinical trials, have already been reported on the wound healing potentiality of aloe gel, as well as on its various components such as polysaccharides, phytochemical constituents such as Aloin A and B, emodin, and acemannan, etc. of this plant [9]. Apart from ethno pharmacological and traditional uses, these studies provide substantial scientific evidence that its wound-healing efficacy is due to the synergistic action of several bioactive components rather than any single component [10].

Nanotechnology is a rapidly growing and complicated research topic, scientists are interested in a variety of biomedical applications [11]. Only a few of the domains of life where nanoparticles have been used are industry, health, food, biomedicine, and cosmetics. A material particle having a diameter of one to one hundred nanometers is considered a nanoparticle or ultrafine particle according to conventional definitions (nm). There are many applications for nanoparticles in life, including the medicinal, cosmetics, food, and industrial fields. ZnO-NPs are the most intriguing inorganic metal oxides due to their simplicity of preparation, low cost of production, and cleanliness [12]. Numerous studies have been done to show how well zinc works to heal wounds. It has been demonstrated that preparations containing zinc accelerate the healing of wounds and reduce bacterial infection at the tissue site. Additionally, they helped the wound's granulation tissue to develop [13]. ZnO is an inorganic substance that demonstrates exceptional qualities such as semiconductor, broad radiation absorption, piezoelectric, pyroelectric, and high catalytic activity. Nanoparticles are being used in a variety of areas of life, including the biomedical, cosmetics, food, and industrial sectors. The most intriguing inorganic metal oxides are ZnO-NPs because they are easy to prepare, affordable to produce, and clean [12]. Recently developed antimicrobial nanoparticles have proven to be quite helpful, especially for wound healing [14]. It has also decreased the price of treatment when

compared to conventional antibiotics. Since studies on their impact on wound healing were conducted, zinc oxide nanoparticles have gained a lot of attention. This research has produced encouraging findings. The efficiency of aloe vera with zinc nanoparticles in rabbits has hardly been studied at all. So, bearing in mind the above mentioned facts, the general objectives of medicinal use of aloe vera gel and ZnO nanoparticles has been determined to asses healing rate, healing time, WBC count on burn wound cause by iron fire in rabbit model.

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, Bangladesh.

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 aloe vera extract, T_2= Hot iron burned and treated with aloe vera extract and ZnO-NPs and T_3= Hot iron burned and treated with commercial drug (Bactrocin®).

2.4 Collection and Preparation of Extract

The Hajee Mohammad Danesh Science and Technology University (HSTU) campus is where the aloe vera leaves were collected. The fresh leaves of Aloe vera were washed carefully with distilled water to remove any extraneous material. Using a small spoon scooped it into your blender carefully not to include any pieces of the Aloe vera skin. Blend the gel until its frothy and liquefied, which should only take a few seconds. The sample preparation was followed by [15].

2.5 Combined Used of Aloe Vera Gel and ZnO-NPs

The preparation was made by combining 4g of aloe vera 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 [16].

2.6 Use of Commercial Drug (Bactrocin®)

As an ointment, Bactrocin® is used in this research. It is used as 1gm/animal twice daily.

2.7 Data Collection

2.7.1 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.

2.7.2 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 [17].

2.7.3 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.

2.7.4 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.

2.8 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 (%)

Table 1 the result represent the healing rate of the hot iron burn wounded rabbits gradually increased from 1st week to 4th week of the study period and showed significant variation among the experimented treatments (P<0.01). The highest healing rate was obtained from T_2 (aloe vera gel with ZnO nanoparticles) treated group (94.75±1.31) followed by T_3 (Commercial Drug Bactrocin®) treated group (92.5±1.32), T_1 (Aloe vera gel) (85.5±2.21) and T_0 (Control) group (70.5±2.72). Amongst the studied treatments the T_2 (Aloe vera gel with ZnO nanoparticles) treated group showed the best performance in relation to healing rate throughout entire the study period (1st week to 4th week). Aloe vera gel can not only increase the amount of collagen in wounds but also change the composition of collagen, increase collagen cross-linking and thereby promote wound healing. ZnO-NPs were a well-known antibacterial agent and effective at very low concentration of bacteria as confirmed by previous studies. In addition, the small size of the biosynthesized ZnO-NPs provides a large surface area and this leads to more contact between the NPs and the bacterial cells. Thus the biosynthesized ZnO-NPs with aloe vera plant gel have been proven to have antibacterial property. This was in accordance with [18] which demonstrated that the biosynthesized against bacteria such as *Staphylococcus aureus, Serratiamarcescens, Proteus mirabilis* and *Citrobacterfreundii* and as well as fungi like *Aspergillusflavus, Aspergillusnidulans, Trichodermaharzianum* and *Rhizopusstolonifera*. These led towards to healing rate than other treatments.

Table 1. Effects of Aloe vera gel, Aloe vera gel with ZnO nanoparticles and Bactrocin® on hot iron burn wound healing rate (%) of rabbit weekly

Treatment	1st Week	2nd Week	3rd Week	4th Week
T_0	25.00±1.58^a	39.75±0.85^a	57.00±1.87^a	70.50±2.72^a
T_1	27.50±1.32^a	47.25±3.35^a	63.25±2.86^a	85.50±2.21^b
T_2	46.25±1.49^c	68.00±1.77^b	84.50±0.64^b	94.75±1.31^c
T_3	39.75±154^b	60.25±2.01^b	81.00±1.58^b	92.50±1.32^{bc}
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 aloe vera extract, T_2= Hot iron burned and treated with aloe vera extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

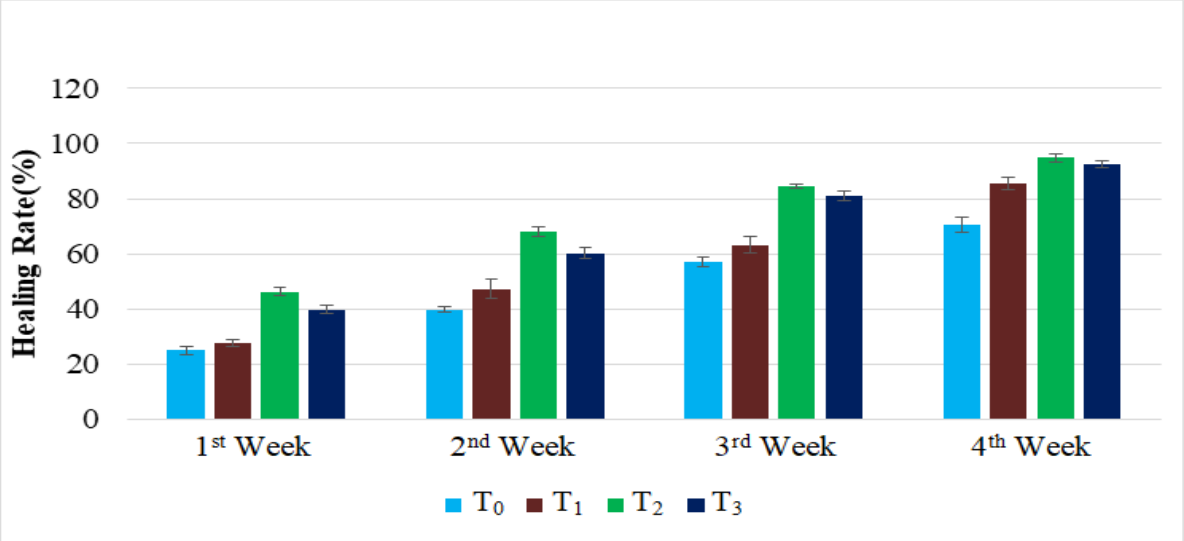

Fig. 1. Effects of Aloe vera gel, Aloe vera gel with ZnO nanoparticles and commercial drug (Bactrocin®) on hot iron burn wound healing rate (%) of rabbit at weekly

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

3.2 Healing Time

Table 2 showed the healing time requirements (days) of the different treatments during the study period. The result of the study showed significant variation among the experimental treatments (P<0.01). The T_2 (aloe vera gel with ZnO nanoparticle) treatments performed best (24.25±0.47 days) which takes minimum days than other treatments to heal the hot iron burn wound of rabbit followed by T_3 (commercial drug Bactrocin®) treatments (26.5±0.64 days) and T_1 (aloe vera gel) treatment (28.25±0.75 days). On the other hand the control group took the maximum days to settle the artificial hot iron burn wound of the rabbits. The ZnO and aloe vera gel where the free carboxylic and the amino group of the plant gel acted as both reducing and capping agent. The possible mechanism of interaction between ZnO ions with plant phytochemical is the repetition of redox reaction during glycolysis process that involves the conversion of carbohydrate to energy. These interactions run into highest and fast healing of burn wound of rabbit [19].

Table 2. Effects of Aloe vera gel, Aloe vera gel with ZnO nanoparticles and Bactrocin® on hot iron burn wound healing time of rabbit at 4th week

Treatment	Healing time (Days)
T_0	36.00 ± 0.40^{c}
T_1	28.25 ± 0.75^{b}
T_2	24.25 ± 0.47^{a}
T_3	26.5 ± 0.64^{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 aloe vera extract, T_2= Hot iron burned and treated with aloe vera extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

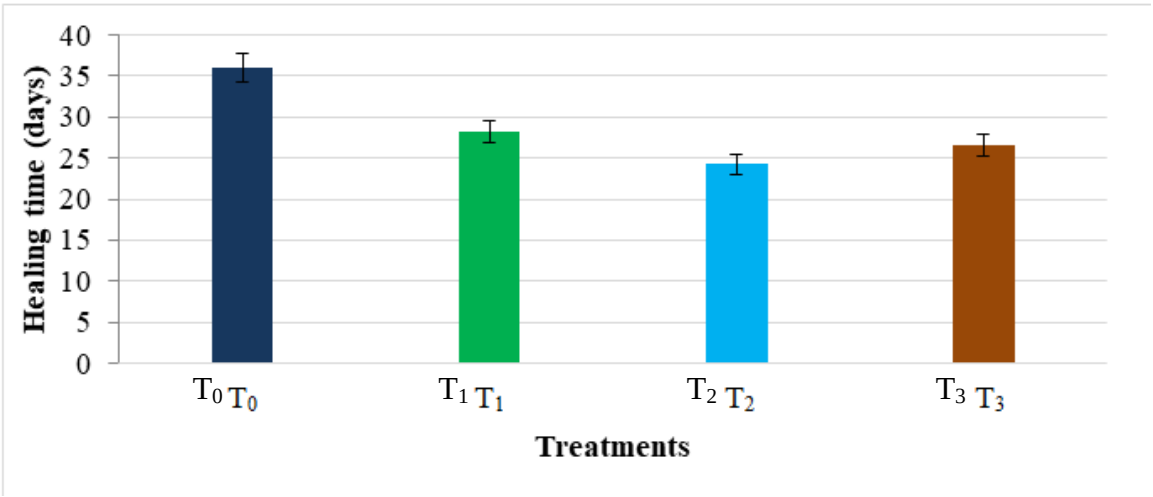

Fig. 2. Effects of Aloe vera gel, Aloe vera gel with ZnO nanoparticles and Bactrocin® on hot iron burn wound healing time (days) of rabbit at 4th week

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

Table 3. Effects of Aloe vera gel, Aloe vera gel with ZnO nanoparticles and commercial drug (Bactrocin®) on WBC (cells/µl) count of rabbit at weekly interval

Treatment	Day 0	1st week	2nd week	3rd week	4th week
T_0	5700 ± 30.85^{c}	9075 ± 36.45^{b}	7800 ± 35.48^{c}	6500 ± 41.01^{ab}	6000 ± 33.69^{b}
T_1	5200 ± 41.20^{b}	8900 ± 27.36^{b}	7500 ± 25.97^{b}	6400 ± 34.25^{a}	5300 ± 40.05^{a}
T_2	4800 ± 36.25^{a}	8000 ± 29.01^{a}	7200 ± 42.3^{a}	6500 ± 39.26^{ab}	5400 ± 29.45^{a}
T_3	5100 ± 35.25^{b}	9300 ± 37.50^{c}	8500 ± 37.36^{d}	6600 ± 24.25^{c}	5400 ± 31.47^{a}
P-Value	0.000***	0.000***	0.000***	0.035*	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 aloe vera extract, T_2= Hot iron burned and treated with aloe vera extract and ZnO-NPs, T_3= Hot iron burned and treated with Bactrocin®

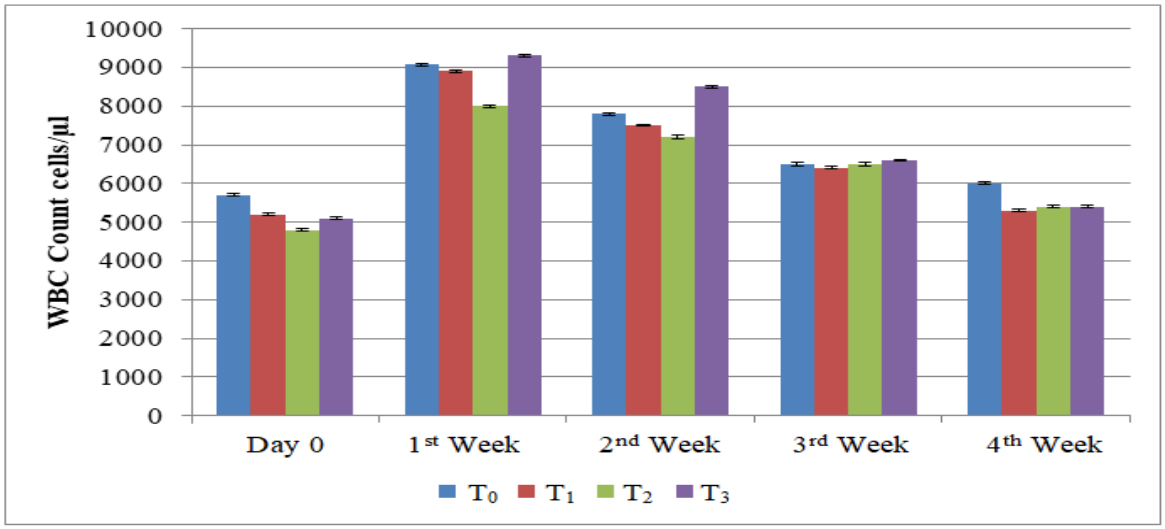

Fig. 3. Effects of Aloe vera gel, Aloe vera gel with ZnO nanoparticle and commercial drug (Bactrocin®) on WBC count (cells/µl) of rabbit at weekly interval

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

Table 3 above showed that the WBC count of the different studied treatments which was significantly influenced by the different experimented groups (P<0.01). The present study revealed that the WBC count was greatly increased in the non-treated group/control group (at 0 and 28 days) which is (5700±30.85 and 6000±33.69 cells/µl respectively). At day 7, 14, 21 and 28 the maximum WBC count was counted on T_3 (9300±37.50, 8500±37.36, 6600±24.25 and 5400±31.47 cells/µl respectively). The lowest WBC count was obtained from T_2 treated groups in most of the cases. The results showed that the (Bactrocin®) application improve the WBC count than the other treatment group [20].

4. CONCLUSIONS

The results of the study showed that aloe vera gel with ZnO nanoparticles resulted in faster wound healing with a lower minimum day's requirement, demonstrating that wound healing was still possible without the use of antibiotics like Bactrocin®.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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