The combined effects of Aloe vera gel and silver nanoparticles on wound healing in rats

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ABSTRACT

Objective(s): This study was aimed at investigating the synergy effects of Aloe vera gel and silver nanoparticles on the healing rate of the cutting wounds.

Materials and Methods: In order to determine the concentration of silver nanoparticles in Aloe vera gel, the MBC methods were applied on the most common bacteria infecting wounds, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa. The cutting wounds with Full-thickness skin were dorsally created on rats; then the rats were divided into 4 groups. The treatments groups included: mixture of Aloe vera gel and silver nanoparticles, Aloe vera gel alone and silver nanoparticles alone in addition to control groups. The treatment was carried out for 2 weeks and the size of the wound closures were measured by an image software analysis.

Results: There was no significant difference (p<0.05) in healing rate between the control and mixture group. However, there were significant differences between the silver nanoparticles and Aloe vera groups using Tukey’s analysis on the 6th, 8th and 10th days.

Conclusion: The Aloe vera gel increased the rate of wound healing whereas the silver nanoparticles had a delay effect; and when they were mixed, it was similar to the average effect of both Aloe vera gel and silver nanoparticles.

Keywords: Aloe vera gel, Silver nanoparticle, Wound healing

INTRODUCTION

Increasing the rate of wound healing in burns and skin injuries has always been of great interest to medical professions. Wound healing is a complex process by which shearing wounds and burn wounds all heal through the same process [1]. Shear-type wounds, has little effect on the skin and may lead to epithelial and connective tissue cells with less interference to the integrity of the epithelial basement membrane. This is called a granulation in order to improve the original or primary. Several factors are causing delays in wound healing. Insufficient nutritional resources available to the skin cells, dysfunctional immune system and presence of an infection which may be the normal flora or other opportunistic microbes that are in the environment. The most common infections in the wounds are caused by Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus species and Escherichia coli [2]. Different course of antibiotic resistance, especially in methicillin resistant Staphylococcus aureus (MRSA), methicillin resistance Staphylococcus epidermidis (MRSE) and vancomycin resistant demand a new approach to management of innovative treatments[3].

Aloe vera leaves contain two products. One is the gel, in the inner side of the leaf and the other is the bitter yellow juice on the outside of inner layer before the external covering. Aloe vera gel is composed of 98.5 percent water and it’s viscous (gel form) due to the sugar of glucomannan. This gel has about 200 active ingredients including minerals, vitamins, proteins, lipids, amino acids and polysaccharides [4]. These compounds can be noted like acemannan which
strengthens the immune system and brady kinase that has anti-inflammatory properties and magnesium lactate that reduce itching and the other soothing and anti-inflammatory such as sialic acid and antiprostaglandins [5]. The yellow juice contains anthraquinone glycosides like Aloin, Aloe-emodin, Barbaloin which are potentially laxative. Effect of Aloe vera gel for wound healing is to modulate and optimize the immune system to accelerate wound healing process [6]. Aloe vera shows a beneficial effect by reducing the inflammation significantly and providing a more mature granulation tissue which could accelerate healing and might produce a sound well-remodeled scar [7]. It increases the activity of macrophages and monocytes and stimulation of killer T lymphocytes which is done through releasing the interleukin 1 and 6, and TNF-α, INF-β. Aloe Vera gel can also block the production of prostaglandin and thromboxane from arachidonic acid to reduces inflammation in the wound [8]. It can also have inhibitory effect against pathogenic bacteria, causing food poisoning or different diseases in humans [9]. There are about 200 species of Aloe vera in the world. Among all, Aloe vera Barbadensis Miller is the best-known because of being the richest in useful materials for healing [10]. Nowadays, because of its antimicrobial activity, the use of silver nanoparticles (SNPs) has become very widespread [11]. Nano silvers are of various morphologies including spheres, rods, cubes, triangles, wired and multifaceted that they all have a size less than 100 nm. Due to their shape, surface charge, the surface-to-volume condensation, integrated power and other factors, their behavior will vary with their surroundings [12]. The possible mechanism of antimicrobial activity is due to several effects. SNPs interact with proteins and enzymes of thiol groups [12], and disrupts processes such as cell respiration, ion transport across membranes [13]. The ATP production and the ability of the replication of DNA [14] and block activity of microorganisms. However, the mechanisms of toxicity to environment are not very well specified [15]. We were interested in investigate the effects of Aloe vera gel and antibacterial properties of SNPs simultaneously together for wound healing in rats.

**MATERIALS AND METHODS**

**Preparation of Aloe vera gel by filleting technique**

Mature leaves of Aloe vera Barbadensis Miller were obtained from a greenhouse of medicinal plants investigation center of jahad university of Kashmar. Gel of Aloe vera was purified by filleting technique [16]. After washing and drying the leaves, two cuts were created parallel to the longitudinal leaf using a scalpel at the edges of leaves. Cutting the veins in the transverse direction leads to a combination of bitter yellow juice with Aloe vera gel that is not of our interest. The leaf epidermis was removed and gel from the leaf cavities was shaved by a sterile spoon and then transferred to a sterile blender and was mixed for 5 minutes. By mixing the cells, they rupture and their content are released. Then we wait at least for 15 minutes until the foam reduced and suspended chopped cell pieces precipitated. Having used a glass funnel and sterile gauze with holes which can take carcass of the cells, the gel was filtered into a sterile flask. Then it was distributed in volumes of 50 ml in sterile plastic containers with lid and were kept in a freezer (-20°C) for later use.

**Determination of concentration of SNPs in Aloe vera gel**

Silver nanoparticles powder was purchased from Nanostructured & Amorphous Materials (Houston, TX, USA). These SNPs had 20 nanometers (nm) in size, -19 millivolts (mv) in zeta potential, 18-22 gr/m2 in surface area and hydrophilic surfact by Poly vinil pirolidone 0.3%. A stock concentration (1000 µg/ml) was made from the silver nanoparticles in sterile distilled water by 10 minutes sonication for next applications. To determine the minimal bactericidal concentration (MBC) of SNPs with the least toxicity effects of them, it was decided to employ the most common bacteria causing wound infections: Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922. Half concentration McFarland of fresh bacteria in 0.09 % sterile saline which was equivalent 1.6×106 bacteria per ml was made. Aloe vera gel with consecutive concentrations of SNPs in sterile tubes was prepared and 10 microliters of bacterial suspension equal to 1.6x104 bacteria was added to 1ml of each sterile tubes, approximately 1.5 million bacteria. The lid tubes were shaken and incubated at 37°C for 24h. The next day, from each tube, a small amount of contents was transferred to blood culture medium [17, 18]. With the addition of 10 µl bacterial suspension to 1ml dilution tubes, 1% error is caused which is corrected in the dilution. Culture results in prepared dilutions are shown in Table 1.
Creating wounds and selection of the treatment groups of rats

This study was conducted on young male Wistar rats aged 8 to 10 weeks and weighing 250 to 300 g. Rats were adapted to room conditions after being transferred, where they were kept in individual cages for 24 hours. Special conditions of ventilation, temperature, lighting, humidity, nutrition and safe drinking water were considered to all the same until the possible influence of environmental factors in wound healing between the groups be equal. After a day, the rats were adapted to the conditions in the room for a second day, then the rats were anesthetized for a time of food deprivation and their hairs between the animal’s shoulders were shaved dorsally on the surface. An incision with length of 3 centimeters (cm) and depth of full-thickness on shaved dorsal surface between the animal’s shoulders was made so that the incision are not available to the animals. In this kind of wound both the epidermis and dermis layers were damaged. Wound areas were nearly 3.7 cm\(^2\) on 0\(^{th}\) day.

The animals were randomly divided into 4 groups of 7 rats in each. The control group was (A or control) treated with normal saline, the SNPs group was (B or SNPs) treated with 20 µg/ml of SNPs in sterile distilled water, The Aloe vera group (C or Alo) treated with Aloe gel and the mixture group (D or SNPs-Alo) which was treated with 20 µg/ml of SNPs in Aloe vera gel. During the process of treatment, standard maintenance for rats was done under the Animal Experiment Regulations.

Table 1. The MBC determination of different dilutions of silver NPS

<table>
<thead>
<tr>
<th>Density (µg/ml)</th>
<th>S. aureus</th>
<th>E.coli</th>
<th>P.aeroginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>500</td>
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<td>100</td>
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<td>50</td>
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<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control+ (10000)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control – (0)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

SNPs in gel was prepared. To study the dispersion of the SNPs in Aloe vera gel, Transmission Electron Microscope images were taken as shown in Fig 1.

Treatment of wound area

The rats were received treatment by seeping the lotion on the wound twice daily, once 0.5 ml and then 1 ml. Wounds area measurements were done on even days in terms of square centimeter (cm\(^2\)) with using a high-quality digital camera and Image Analysis Software (Digimizer.v4.1.1.0).

By a scale ruler four photos were taken from each wound and was used to measure average. Information related to reduction of wound area and their measurements are shown in Table 2. Also Percentage of Wound Healing (PWH) was calculated which are shown in Table 3. PWH was calculated by the following formula.

\[
\text{Percentage of wound healing (PWH) on } \chi \text{ day} = 100 - \left( \frac{\text{Percentage of wound on } \chi \text{ day}}{\text{Wound area on zero day}} \right) \times 100
\]

When the wound area was reduced to zero (full wound closure) and full recovery was achieved, randomly 3 samples of each group from the improved wound skin were biopsied up for histological examination. Samples were stained by Hematoxylin-Eosin staining, and they were evaluated in terms of factors Table 4.

Statistical analysis

The calculations were performed using IBM SPSS Statistics 19 software. To determine differences of reduction of the wounds area between groups and assessing their improvement, ANOVA and Tukey’s test with a significance level of p<0.05 were used with control and two by two.
The combined effects of Aloe vera gel and silver nanoparticles on wound healing

Fig. 1. TEM images of SNPs present in gel of Aloe vera

Table 2. Reduction of wounds area on different days

<table>
<thead>
<tr>
<th>Groups</th>
<th>n=7</th>
<th>Woundarea on day 0</th>
<th>Woundarea on day 2</th>
<th>Woundarea on day 4</th>
<th>Woundarea on day 6</th>
<th>Woundarea on day 8</th>
<th>Woundarea on day 10</th>
<th>Woundarea on day 12</th>
<th>Woundarea on day 14</th>
</tr>
</thead>
</table>
| Control A | 3.729±0.36 | 2.854±0.29 | 1.669±0.24 | 0.855±0.12 | 0.478±0.08 | 0.201±0.1 | 0.087±0.07 | 0.008
| SNPs B | 3.779±0.1 | 2.949±0.19 | 1.791±0.33 | 1.041±0.18 | 0.628±0.11 | 0.278±0.06 | 0.075±0.4 | 0.006
| Alo C | 3.718±0.17 | 2.583±0.12 | 1.473±0.23 | 0.608±0.13 | 0.295±0.13 | 0.074±0.03 | 0.001±0.03 | 0
| SNPs-Alo D | 3.691±0.29 | 2.584±0.31 | 1.493±0.26 | 0.848±0.26 | 0.366±0.16 | 0.169±0.06 | 0.057±0.05 | 0.003

Table 3. Percentage of wound healing (PWH) on different days

<table>
<thead>
<tr>
<th>Groups</th>
<th>n=7</th>
<th>PWH on day 0</th>
<th>PWH on day2</th>
<th>PWH on day4</th>
<th>PWH on day6</th>
<th>PWH on day8</th>
<th>PWH on day10</th>
<th>PWH on day12</th>
<th>PWH on day14</th>
</tr>
</thead>
</table>
| Control A | 0 | 22.6±0.29 | 54.9±0.28 | 76.9±0.11 | 87±0.09 | 94.5±0.1 | 97.7±0.08 | 99.9
| SNPs B | 0 | 22±0.18 | 52.7±0.31 | 72.5±0.16 | 83.4±0.12 | 92.7±0.07 | 97.2±0.5 | 99.7
| Alo C | 0 | 30.5±0.15 | 60.4±0.21 | 83.6±0.15 | 92.1±0.1 | 98±0.04 | 100 | 100
| SNPs-Alo D | 0 | 29.7±0.28 | 59.4±0.27 | 77±0.24 | 90.1±0.14 | 95.4±0.08 | 98.5±0.06 | 99.9
RESULTS

When SNPs mix with Aloe vera gel, probably due to inorganic and organic compounds in gel and changes in surface charge of them, some of SNPs can stick together which seen in Fig 1. But antibacterial effects of SNPs were evaluated on this gel. Therefore, in order to determine the concentration of SNPs in Aloe vera gel, MBC method was used which is shown in Table 1. Thus, the concentrations of 20 g/ml SNPs in Aloe vera gel were used to prepare the mixture gel. By this method, it has been shown that each 1 ml Aloe vera gel containing 20 μg SNPs (20 nm) can nearly kill 1.5 million bacteria. This number of bacterial has been selected as a default from infection. On the other, because of toxicity in SNPs, high concentrations of SNPs could not be used. Table 2 shows reduction of wounds area on even days. Wounds area were nearly 3.7 cm² on 0th day. Table 3 shows PWH on even days which they are 0 % on 0th day. Figs 2 and 3 show the data of Table 2 and 3. All the groups were compared statistically with the control group and two by two. Aloe group (C) was treated 2 days earlier than the control group (A) in 12th days. SNPs-Alo group (D) and control group (A) were treated at the same time in 14th days. SNPs group (B) was treated later than other groups in 15th days. The rate of healing between groups were Alo (C) > SNPs-Alo (D) = Control (A) > SNPs (B). In order to better compare the curves in Figs 2 & 3 and verification accelerate wounds healing between groups, trendlines were used. There is no a significant difference (p<0.05) in wound healing between control (A) and SNPs-Alo (D) groups, but there are significant differences between SNPs (B) and Aloe (C) groups on days of 6, 8 and 10 as well as between control (A) and Aloe (C) groups on days 10 and 12. There is also a significant difference between SNPs (B) and SNPs-Alo (D) groups on day 8. After full recovery, 3 rats were randomly selected and samples were taken for histological testing. Table 4 shows histological hemosiderin deposition and formation of granulation tissue and fibroblasts occurred in tissue formation equally in all groups. But it shows differences between their epithelialization processes. It was complete in Aloe (C) and control (A) groups and there isn’t in the SNPs (B) group and nearly complete or defect in the SNPs-Alo (D) group. Fig 4 is optical microscopy images of cells of wounds which stained with Hematoxylin-Eosin. During treatment and healing of wounds, ulcers abnormalities were not observed and all rats were treated in the normal way.

DISCUSSIONS

Fig1 shows the transmission electron microscopy images of Aloe vera gel containing SNPs indicating the aggregation of SNPs. This means that Aloe vera gel has the potential to aggregate SNPs together that it could be due to changes in surface charge of them. The charged or non-charged amino acids, organic acids, mineral elements that had participated in the aloe gel such as arginine, asparagine, glutamic acid, aspartic acid, salicilic acid, calcium, iron, zinc, copper and else [19, 20].
Fig. 2. Reduction of wounds area from 3.7 cm\(^2\) to zero on even days with trendlines

Fig. 3. Percentage of wound healing (PWH) from 0 to 100\% on even days with trendlines

Fig. 4. Optical Microscopy images of cells obtained from wounds and stained with Hematoxylin-Eosin
It is certain that MBC test is done by the same mixed gel with connected particles. Result of this research shows that the Aloe vera gel accelerates wound healing, as in several studies have been investigated [21-24]. In several studies, good results have been taken from the SNPs along with other materials [7, 25-28]. However, this study shows that SNPs partly reduce rate of wound healing especially alone. It can be due to several effects of SNPs that interact with cells they are recovering. Asha Rani & et (2008) in a study on the toxicity of SNPs on the genome of human cells to conclude that SNPs enter to the cell and the nucleus and binding to the genetic material and they arrest cell cycle in the G2 phase of mitosis which can cause damage to cells, So it is possible that the lack of epithelialization in the SNPs (B) group is due to this action [29].

This means that toxicity effects of SNPs on the proliferation of epithelial cells in mitosis lead to the delayed recovery. According to Table 4, only complete epithelialization process can be observed in the control (A) and Aloe (C) groups, it is not formed in the SNPs (B) group and it was on average defective in the mixed (D) group. Chen et al [12] in 2008 in a study on the toxicity of SNPs concluded that SNPs cause a sharp decrease in mitochondrial activity and lead to cell malfunction. It has been found that silver ions seems to perturb mitochondria through interactions with thiol groups of the mitochondrial inner membrane. As these effects of silver ions could be completely blocked by sulphhydryl reagents, (e.g. reduced glutathione (GSH)), the findings clearly suggested that mitochondria were under oxidative stress when the cells were exposed to silver ions [12]. In conclusion, a preliminary outcome of this study could be that SNPs may interact with proteins and enzymes containing thiol groups such as glutathione, thioredoxin, SOD and thioredoxin peroxidase, are key components of the cell’s antioxidant defense mechanism and are responsible for neutralizing the oxidative stress of ROS largely generated by mitochondrial energy metabolism.

SNPs may deplete the antioxidant defense mechanism which leads to ROS accumulation. Accumulation of ROS could initiate perturbation and destruction of the mitochondria as shown in Table 4 [12, 30]. According to the investigated subjects, SNPs do not appear to increase wound healing as obtained in this study. In fact, it could be suggested that SNPs have delayed wound healing process. In the case of epithelialization defects in group treated with SNPs-Alo mixture, it could be suggested that the Aloe vera gel increased the rate of wound healing whereas SNPs decrease it, but the mixture of SNPs and Aloe gel has produced an effect which was the sum of each component of the mixture as shown in Figs 2 and 3. Perhaps the mixed gel had no effect on recovery, however, it is recommended for cases such as topical use in wound with severe infection, in patients with immunodeficiency who have wound or want to prevent ulcer formation such as Diabetic Foot, drug resistances and development of potential anti-bacterial biofilm for various biomedical and environmental applications against MRSA and MRSE [26].

CONCLUSION

It could be concluded that Aloe vera gel is effective in speeding up the wound healing process but when mixed with SNPs, no significant improvement was observed in the healing rate.

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