RESEARCH ARTICLE

Synergistic antimicrobial activity of honey combined with silver nanoparticles topically applied in experimentally infected wounds in Wistar albino rats

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ABSTRACT

Background: Silver nanoparticles (AgNPs) show ample antibacterial activities when combining with honey. Aim and Objective: The study was designed to determine the antibacterial activity of different kinds of honey against the common wound pathogen Staphylococcus aureus. Materials and Methods: Bacterial strain was used to inoculate the wound. S. aureus ATCC-25923 was used in the study. Results: Wound infection examination was done by total count of bacterial load. In all three groups, better percentage of the wound closer, histological, blood, and tissue parameters identified in honey combine with AgNPs, showed a synergistic effect. Conclusion: Honey and AgNPs showed synergistic effect. It is recommended to apply both the products together.

KEY WORDS: Honey; Silver Nanoparticles; Staphylococcus aureus; Blood and Tissue Parameters

INTRODUCTION

Management of wounds is a herculean task till date by the clinical point of view. There are many agents that can give relief but at same time associated with side effects also. Hence, the natural product use was recommended in this wound management.[1-3] Further, the amount of money involved in the treatment is a big deal in the management of wound. Especially in country like India, people cannot afford costly drugs. Hence, natural remedies which are cheaper and also effective are encouraged. Hence, it is a topic of interest to most researchers to develop cost-effective and efficient management of wounds.[4] The healing of a wound occurs in four phases that are hemostasis, inflammation, proliferation, and maturation.[5] Various growth factors were found to be necessary for the initiation and promotion of wound healing.[6] Earlier studies reported positive benefits of use of honey in multiple clinical disease management.[7] It has antibacterial action and kills the bacteria effectively.[8] This function of honey is very valuable because when wound happens, the bacteria will start growing in the wound and makes it delay in cure.[9,10] When honey is applied and it can be able to deal with the bacteria, the healing process will be speeded up.[11] Nanoparticles use was greatly increased in current context.[12] When compared to other nanoparticles, silver was reported to
be more effective when used for healing the wound.\cite{13,14} It has antibacterial properties which made it more effective agent in the management of wounds.\cite{15} The current study observed the synergistic antimicrobial activity of honey combined with silver nanoparticles topically applied in experimentally infected wounds in Wistar albino rats.

**MATERIALS AND METHODS**

**Study Setting**

The study was conducted at the Department of Research and Development, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India.

**Experimental Animals and Study Design**

Sixteen healthy adult male Wistar albino rats weighing 250–300 g were used in this study. Randomly selected rats were assigned to 3 (1–3), control group contains four rats remaining two groups of six rats each.

**Experimental Wound Preparation**

An excision wound model was used for this study. A wound of 1.5 cm was created using a sterile scalpel by removing the skin on the dorsum of the abdomen of all groups\cite{16} under isofluorane anesthesia. The wounds were infected by instilling 100 μl strains of Staphylococcus aureus. Evaluation every five days, i.e., on days 1, 5, 10, and 15.

**Measurement of Wound Infection**

Wound infection was assessed using measurement of bacterial load at the wound site on days 1, 5, 10, and 15 days swab test was performed from the wound surface for analyzing the superficial load of bacteria

**Procedure**

The untreated group was left without any treatment to serve as the untreated group 1 (control group), group 2 (positive control) was treated with honey topically 0.5 ml/cm², while group 3 (experimental group) was treated with 0.04 mgkg⁻¹ silver nanoparticles combined with 0.05 mL/cm² of honey\cite{17}

**Rate of Wound Contraction**

The percentage of wound contraction was calculated by dividing the difference in wound area of a particular day from the 1st day by the 1st day area and then multiplying it by a hundred.\cite{18}

**Histological Evaluation**

Excised tissue was fixed in 10% formalin before histological processed. Sections were made and stained with hematoxylin and eosin (H and E) stain. Histological features were observed under 40x of a microscope on days 5, 10, and 15, respectively.

**Blood Collection**

On 5 and 20 days, 1 mL of blood was withdrawn aseptically from the orbital sinus under isoflurane anesthesia and the biochemical parameters were carried out.

**Biochemical Parameters in Blood**

All the biochemical parameters were estimated using a Modular P100 autoanalyzer.

Estimation of albumin: Bromocresol method was used for the estimation of albumin.\cite{19} Estimation of Glucose: Orthotoluidine method was used for the estimation of glucose.\cite{20} Estimation of cholesterol: Trinder’s method was used for the estimation of cholesterol.\cite{20} Estimation of bilirubin: Diazo method was used for the estimation of bilirubin.\cite{20}

**Estimation of Connective Tissue Parameters**

Approximately 250 mg of wet tissue was dried at 50°C for 24 h. It was weighed and kept in glass stoppered test tubes. The final hydrolysate was used for the estimation of hydroxyproline, hexosamine, and hexuronic acid following the standard curve prepared using the proper substrate.

**Estimation of Skin Protein**

On the post-wounding day 18, the protein content of skin tissues was determined by the method of Lowry et al.\cite{21}

**Estimation of Skin Hydroxyproline**

Estimation was performed by standard method specified in the literature.\cite{22}

**Estimation of Hexosamine (HXA)**

Hexosamine content of the samples was determined from the standard curve prepared with D (+) glucosamine hydrochloride (HiMedia Laboratories Pvt. Ltd., Mumbai, India), from 5 to 50 μg/0.5 mL using 100 μg/mL working solution.\cite{23}

**Estimation of Hexuronic Acid (HUA)**

Hexuronic acid content of the samples was determined from the standard curve prepared with D (+) Glucurono-6, 3-lactone (HiMedia, Mumbai, India), from 5 to 40 μg/0.5 mL using 100 μg/mL working solution.\cite{24}
Ethical Consideration
The study protocol was approved by Institutional Animal Ethics Committee (Vide Ref. no SU/CLAR/RD/008/2016).

Statistical Analysis
It is done with one-way ANOVA with Student-Newman-Keuls multiple comparison test. \( P < 0.05 \) was considered statistically significant.

RESULTS

Wound Size
The percentage of wound closure in all the three inoculated groups was almost the same in the beginning. Table 1 given the Mean ± SD of on day 5 percentage of wound contraction 2.5 ± 2.8 is for the control group, 20.4 ± 4.47 is for treated with the honey group, and 36.6 ± 4.08 is for treated with honey combined silver nanoparticles (H+AgNPs), on day 10, percentage of wound contraction 12.5 ± 2.9 is for the control group, 40.5 ± 5.5 is for treated with honey, and 63.33 ± 4.08 is for treated with honey combined silver nanoparticles (H+AgNPs), pairwise comparison honey combined with silver nanoparticles (H+AgNPs) and control, on day 15, percentage of wound contraction 20 ± 4.08 is for the control group, 65 ± 5.84 is for treated with honey, and 68.3 ± 4.08 is for treated with honey combined silver nanoparticles (AgNPs), and on day 20, percentage of wound contraction 32.5 ± 6.45 is for the control group, 85.8 ± 3.8 is for treated with honey, and 90 ± 3.16 is treated with honey combined silver nanoparticles (AgNPs). In all three groups, better percentage of the wound closer identified in honey combine with silver nanoparticles (H+AgNPs), on day 10, 20 ± 4.08 is for the control group, 40.5 ± 5.5 is for treated with honey, and 63.33 ± 4.08 is for treated with honey combined silver nanoparticles (H+AgNPs) treated groups total protein showed significant with control, honey and honey combined with silver nanoparticles (H+AgNPs) treated group’s blood glucose levels showed significant with \( P < 0.05 \) on the 5th day and 20th day [Table 2].

Histopathological Observations
In all three groups of animals, wound sections showing ulceration and intense inflammation, edema treatment not yet started, initial stage of angiogenesis, showing subepithelial sections showed lag in granulation tissue formation, thin layer of epithelization, and granulation tissue formation in control group sections on day 15 lag in reepithelization, continuing reepithelization, and collagenous matrix formation and complete closing of the wound in honey combine with AgNPs [Figure 1].

Biochemical Parameters in Blood
Biochemical parameters such as albumin, blood glucose, cholesterol, and bilirubin were analyzed. When compared with control, honey and honey combined with silver nanoparticles (H+AgNPs) treated group’s blood glucose levels showed significant with \( P < 0.05 \) on the 5th day and 20th day [Table 2].

Estimation of Connective Tissue Parameters
Tissue parameters such as total protein, hydroxyproline, hexuronic acid, and hexosamine were analyzed. When compared with control, honey and honey combined with silver nanoparticles (H+AgNPs) treated groups total protein and hydroxyproline levels showed significant on the 5th day and 20th day [Table 3].

DISCUSSION
The current study observed the synergistic antimicrobial activity of honey combined with silver nanoparticles topically applied in experimentally infected wounds in Wistar albino rats. Honey and AgNPs showed synergistic effect. It is recommended to apply both the products together. Honey is natural product which is available throughout India. The health benefits of honey were well documented. Along with its regular beneficial effects, honey was reported to have a role in the management of the wounds. It mainly speeds up

Table 1: Percentage of wound contraction of control, honey, and honey+AgNPs S. aureus inoculated wound model statistical analysis is done with one-way ANOVA with Student-Newman-Keuls multiple comparison test. \( P<0.05 \) was considered statistically significant where \( n=4 \) in control and positive control and \( n=6 \) in experimental group (Mean±SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group without any treatment</th>
<th>Positive group treated with honey</th>
<th>Experimental group treated with honey+AgNPs</th>
<th>Statistical analysis</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5th day</td>
<td>2.5±2.88</td>
<td>20±4.47</td>
<td>36.6±4.08</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>10th day</td>
<td>12.5±2.9</td>
<td>40.5±5.5</td>
<td>63.3±4.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>20±4.08</td>
<td>65±5.84</td>
<td>86.6±4.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20th day</td>
<td>32.5±6.5</td>
<td>85.8±3.8</td>
<td>90±3.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( S. \) aureus: Staphylococcus aureus

Table 2: Hematological parameters on control, honey treated, and honey+AgNPs, S. aureus inoculated wound (Mean±SD) (\( n=4 \-6 \)).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Day</th>
<th>Groups</th>
<th>Parameters Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S. ) aureus</td>
<td>5</td>
<td>Control</td>
<td>2.5±0.19  67.2±1.7  54±1.6  0.27±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honey</td>
<td>2.4±0.09  66.6±1.6  53.5±1.5  0.22±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H+AgNPs</td>
<td>3.3±0.15  74±8±0.9  47.3±1.6  0.29±0.01</td>
</tr>
<tr>
<td>( S. ) aureus</td>
<td>15</td>
<td>Control</td>
<td>3.4±0.17  84.5±0.9  54.5±1.9  0.20±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honey</td>
<td>3.3±0.20  85.6±1.5  58±1.78  0.11±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H+AgNPs</td>
<td>3.4±0.20  93.8±2.4  39±2.09  0.24±0.01</td>
</tr>
</tbody>
</table>

\( S. \) aureus: Staphylococcus aureus
the healing process by reducing or limiting the growth of the bacteria in the wound. This has immense role as the bacterial growth always increases the infection and delays the healing process. Hence, the honey has a pivot role in the management of wound healing. Use of the nanoparticles was increases in recent years as they have multiple beneficial effects including management of certain diseases also. One of the immense nanoparticles is silver. Silver has multiple beneficial effects and it has a role in wound healing. The antibacterial functions of silver make it possible to take part in speeding up the process of wound healing. The special characteristic of silver is that it can acts on any type of bacteria that means even it can kill bacteria that has resistance with available antibiotics. Earlier studies explained and proved the bactericidal effects of silver. It was reported that silver has effective action to kill the bacteria that are commonly present in the wounds and cause minimum side effects. There was no reported harmful effects of silver till date.

Silver also being used as a preservative substance for certain chemicals. It was reported that silver increases the healing process and speed up all the steps involved in the healing.

Further wound contraction which is very essential in the healing process also speedup by the silver nanoparticle. The present study investigated the together effects of silver and honey and it was found that it was very effective when they applied together rather applied alone. The study recommends detailed studies in this area as both the ingredients used in the study were cost effective and have multiple benefits.

CONCLUSION

The study revealed that there exists a synergistic effect of both honey and silver nanoparticles when applied together for healing the wound. The study also recommends further detailed studies in this area to recommend the medical use of these products.

REFERENCES

Antimicrobial activity of combination of honey and silver nanoparticles


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