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# EFFECTIVENESS OF SILVER SULFADIAZINE ON WOUND HEALING AND INFECTION IN SPRAGUE DAWLEY RATS

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## ABSTRACT

**Background:** Surgical Site Infections (SSIs) are postoperative infections categorized into superficial, deep, and organ/ space infections, mostly caused by endogenous bacteria. Silver sulfadiazine as topical antimicrobial are widely used, though its effectiveness in SSIs remains unproven. Current research focuses on its impact on lymphocyte and collagen levels in animal models to better understand its role in SSI management. **Objective:** This study aimed to verify the use of silver sulfadiazine in Sprague Dawley rat models in improving healing process of SSIs. **Methods:** The study used a post-test only control group design to evaluate wound treatments on infected wound in rats. Thirty rats were divided into three groups: negative control (0.9% NaCl), positive control (0.9% NaCl + gentamicin), and treatment (0.9% NaCl + silver sulfadiazine). Standard wounds were treated accordingly and on day 14, tissue samples were analyzed for fibroblasts, neutrophils, macrophages, collagen, and wound diameter. Data were analyzed using One-Way ANOVA or Kruskal-Wallis for significant differences ($p < 0.05$). **Results:** Study showed treatment group had significantly higher fibroblast ($p = 0.04$), neutrophil ($p = 0.00$), and macrophage counts ($p = 0.00$), as well as collagen levels ($p = 0.00$), compared to both positive and negative control group. Wound size in treatment group was also significantly smaller. **Conclusion:** Silver sulfadiazine significant reduced fibroblast, neutrophil, macrophage, and collagen counts, as well as wound size.



## BACKGROUND

Surgical site infections (SSI) occur in the incision area following surgery. The CDC classifies SSI into three types: superficial incisional infections, deep incisional infections, and organ/space infections.[1] An infection is classified as an SSI if it develops within 30 days after surgery, involves the skin, subcutaneous tissue, deeper tissues, or organs, and includes purulent drainage or organisms found at the site. SSI is a common occurrence after surgery and is considered part of nosocomial infections.[1] Between 0.5% and 3% of patients undergoing surgery will experience an infection at the surgical site. SSI is particularly prevalent in developing countries, with an incidence rate of 11.8 cases per 100 surgeries. [2,3] The infection is typically caused by bacteria from the patient's own flora, which colonize the surgical site during the procedure. Several factors contribute to the development of SSI, such as the patient's immune system status, presence of foreign materials, bacterial contamination levels, and the use of antibiotics.[3]

Antimicrobial treatments, both systemic and topical, can be prescribed for patients with SSI.[4] Topical treatments have several advantages, including high drug concentration at the surgical site and fewer side effects. Among the topical agents used for SSI are chlorhexidine gluconate, povidone iodine, and silver sulfadiazine ointment.[4] Although no study has directly compared these treatments for SSI, research on burn wound infections suggests that both silver sulfadiazine and chlorhexidine gluconate are similarly effective in reducing bacterial infections.[5] Silver sulfadiazine is a

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topical antibiotic in cream form that prevents bacterial growth in open wounds. It is widely used to prevent infections in burn wounds and promote healing.[6] The silver ions in silver sulfadiazine interact with nucleophilic amino acids and other protein groups, causing protein denaturation and inhibiting enzymes. Additionally, silver disrupts bacterial membranes and proteins, causing proton leakage and leading to bacterial cell death.[7]

Silver sulfadiazine has potential for preventing SSI, as it is safe and well-tolerated, making it a common choice for burn infection prevention.[4] It is particularly effective in preventing SSI in cardiac and colorectal surgeries, as well as in accelerating the reepithelialization of skin grafts. Several biological markers, such as the number of fibroblasts, neutrophils, macrophages, collagen levels, and wound size, are important indicators in the wound healing process. Fibroblasts are crucial in collagen production, a key component of granulation tissue. Neutrophils are immune cells that help combat infection, while macrophages clear debris and modulate the immune response. Monitoring these parameters helps to understand the dynamics of wound healing and the impact of infection.[8,9] To date, no studies have proven the effectiveness of silver sulfadiazine in SSI. As a result, the researchers are investigating the effects of silver sulfadiazine in Sprague Dawley rat models with surgical site infections, specifically focusing on lymphocyte counts and collagen levels.

## METHODS

This experimental study aims to evaluate the effectiveness of various wound treatments in healing infected wounds using a post-test only control group design. The study involved 30 male adult Sprague Dawley rats, weighing 250±50 grams, with a 2-centimeter full-thickness wound created on their dorsal area. The rats were kept at 28.0±2.0°C with 12-hour light cycles and fed ad libitum. Inclusion criteria were healthy male rats weighing 250±50 grams, while exclusion criteria were rats with deformities, female rats, or those showing weakness. Sample size was calculated using Federer's formula, requiring 5 rats per group, accounting for potential dropouts. The independent variable was the administration of silver sulfadiazine, and the dependent variables included the number of fibroblasts, neutrophils, macrophages, collagen, and wound healing diameter.

Rats were randomly assigned to three groups: negative control (wound care with 0.9% NaCl), positive control (wound care with 0.9% NaCl and gentamicin 0,1%), and treatment (wound care with 0.9% NaCl and silver sulfadiazine 10mg/g). After acclimatization, all groups received standardized wounds and specific treatments. On day 14, tissue samples were collected using punch biopsy to assess fibroblast count, neutrophil count, macrophage count, collagen levels, and wound healing diameter. Data analysis will be conducted using the computerized Statistical Package for Social Sciences (SPSS) v.22.0 and analyzed using One-Way ANOVA (for normally distributed data) or Kruskal-Wallis (for non-normally distributed data). Significant differences ($p < 0.05$) were further analyzed with post-hoc tests or Mann-Whitney U tests, respectively. Results were considered significant if p-values were $<0.05$.

## RESULTS

Fifteen rats were divided into 3 different groups. Data on fibroblasts, neutrophils, macrophages, collagen and wound size were read by two pathologists, then combined into one composite value for each sample. The average number of fibroblasts, neutrophils, macrophages, and collagen obtained in positive control and treatment groups was relatively greater than in the negative control. All dependent variables were normally distributed ($p>0.05$) based on the Shapiro-Wilk normality test. The One-Way ANOVA test showed a significant difference in the mean between at least two groups in all study groups in all variables. The Tukey post-hoc test is described in Figure 1.

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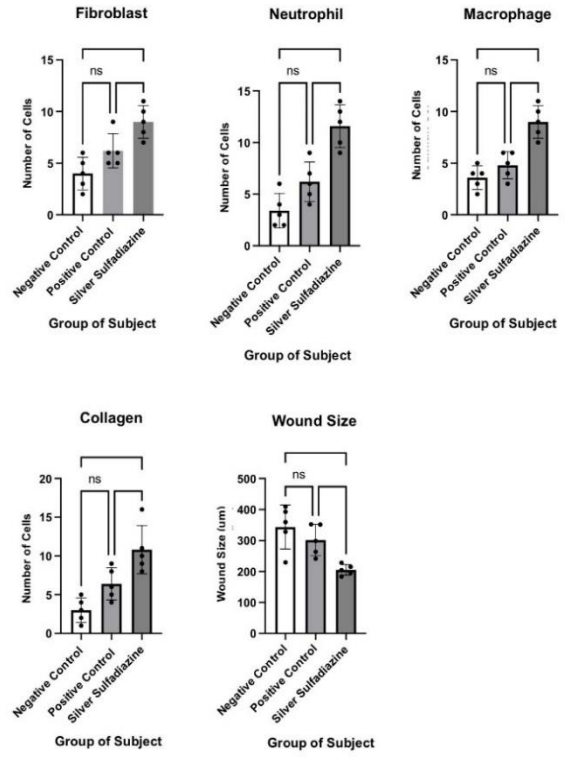


**Figure 1.** Bar chart comparing the number of fibroblasts, neutrophils, macrophages, collagen and wound size

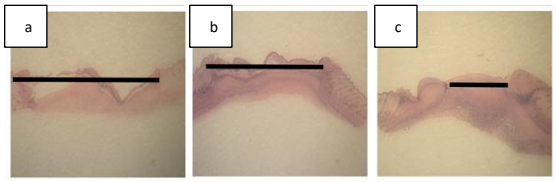


**Figure 2.** Result of wound size from each group before and after intervention; (a) Negative control; (b) Positive control; (c) Silver Sulfadiazine. Wound size treated with silver sulfadiazine is shorter than other groups

## DISCUSSION

### Use of Silver Sulfadiazine

Silver sulfadiazine is commonly used as an antimicrobial agent in wound treatments, especially for burn wounds, which has been proven to reduce infections and accelerate healing.[10,11] This study investigated the effects of silver sulfadiazine on full-thickness wounds in Sprague Dawley rats which showed significant improvement compared to standard treatments, namely gentamicin and saline. The outcome from silver sulfadiazine group can be attributed to its dual mechanism, where silver ions disrupt microbial structures while sulfadiazine inhibits folate synthesis. In addition, silver sulfadiazine ability to downregulate pro-inflammatory cytokines and limit neutrophil infiltration may explain the more organized granulation tissue and faster healing response observed. The slower healing in saline group aligns with literature showing that wounds without antimicrobial or bioactive support are more prone to persistent low-grade contamination and prolonged inflammation. This highlights the importance of early biological control in full-thickness injuries. Recent studies from Ito et al. showed that silver sulfadiazine remains effective even against resistant organisms, which likely contributed to the reduced bacterial load in our full-thickness wounds.[11] Overall, our findings support the broader therapeutic potential of silver sulfadiazine, suggesting that its benefits extend beyond infection control and enhancement of tissue repair.

### Histopathological Outcomes

In this study, the wound treated with silver sulfadiazine had a greater number of collagen, fibroblasts, neutrophils, and macrophages than the positive control group treated with gentamicin and the negative control group treated with saline. Significant differences between the treatment and control groups were validated using a one-way ANOVA analysis. The proliferation of fibroblasts contributes to the formation of collagen, which is essential for wound strength, and the rise in these cell types indicates good wound healing. An aggressive reaction to remove trash and germs is shown by the increase in neutrophils and macrophages.

The effect of silver sulfadiazine on wound healing cells is still up for debate, despite the fact that it is known to have antibacterial qualities.[13] According to certain research, it can be harmful to fibroblasts and interfere with cytokine activation, which could hinder the recruitment of macrophages and delay the healing process.[14,15]

On the other hand, the application of silver ions in Liu et al.'s study positively impacted wound healing by promoting the proliferation and migration of keratinocytes and the differentiation of fibroblasts into myofibroblasts, which enhanced wound contraction.[16] An analysis by Ito et al. showed no significant cytotoxicity in fibroblasts using their silver application method. The success of both studies was based on

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different application methods: nanoparticles in Liu et al.'s research and nanosheets in Ito et al.'s.[17] Cho Lee et al. emphasized the importance of correct silver sulfadiazine dosing to balance antimicrobial effects and minimize cytotoxicity to cells involved in wound healing.[14] In this study, the average number of fibroblasts, neutrophils, and collagen in the silver sulfadiazine-treated group was higher than in both controls, even though silver is known for its cytotoxicity. One-way ANOVA confirmed this difference. This is in line with our findings, suggesting that topical silver sulfadiazine increases fibroblast proliferation and differentiation.[17] Regarding neutrophils, this study's results align with those of Rosen et al., who found silver sulfadiazine increased neutrophils, indicated by higher myeloperoxidase staining in burn wounds after 3 days of application.[18] No studies have addressed collagen deposition with topical silver sulfadiazine, but collagen nanosilver dressings have been shown to increase macrophages and collagen in rat wound models.[19] The increase in neutrophils also boosts macrophage numbers, as demonstrated in studies by Butterfield and Bouchery. Neutrophils are involved in the early phase of inflammation, initiating tissue repair by recruiting macrophages to the damaged tissue. Neutrophils are assumed to play a dual role, clearing pathogens and triggering macrophage recruitment for the next phase of repair (Figure 1). Macrophages repair tissue by producing macrophage-derived MMP9, which recruits more fibroblasts and promotes collagen deposition. In a study by Meschiari et al. and a systematic review by Kloc et al., collagen accumulation enhances tissue remodeling and wound repair.[22-23] Increased collagen synthesis improves wound healing by providing mechanical strength, elasticity, and a substrate for cell adhesion, proliferation, and differentiation.[24]

**Wound Size Outcomes**

The treatment group showed a significantly smaller wound diameter compared to both the positive and negative control groups by the end of the study. The use of silver appears to promote better wound healing. This result is consistent with Liu et al.'s research, which found that silver treatments (including silver sulfadiazine and its nanoparticle form) led to improved wound healing.[17] In contrast, Cho Lee et al. observed slower healing with silver sulfadiazine, likely due to differences in dosage.[14] While gentamicin's effectiveness in preventing surgical site infections has been well established, this study suggests that silver sulfadiazine may provide better wound healing outcomes.[10,11] Treatment with silver sulfadiazine resulted in higher fibroblast counts and smaller wound sizes, indicating its potential in preventing infections and promoting faster healing.[25]

Silver sulfadiazine effectively reduced the risk of infection in our full-thickness wound model by controlling bacterial contamination from the earliest stage, a key factor in preventing SSI-like progression. It created an environment less conducive to colonization while moderating the inflammatory response, as supported by literature showing silver compounds can decrease pro-inflammatory cytokines such as IL-1β and TNF-α. The reduced inflammatory cell infiltration seen in treated wounds reflects a more controlled early phase of healing, allowing timely transition into proliferation. As healing progressed, the continuous antimicrobial support provided by silver sulfadiazine contributed to more robust granulation, angiogenesis, and collagen deposition. Prior studies show that wounds with controlled bioburden exhibit faster fibroblast activity and re-epithelialization, consistent with our histopathological findings. Meanwhile, saline-treated wounds healed more slowly, which aligns with evidence that passive treatments allow persistent low-grade contamination and prolonged inflammation. By the remodeling phase, sustained infection control helped maintain extracellular matrix integrity and prevented delayed healing. Collectively, these results indicate that silver sulfadiazine benefits wound healing not only through antimicrobial effects but by supporting each phase of tissue repair.[25] However, additional research particularly in humans, is necessary to determine if silver sulfadiazine can become a standard treatment for surgical wounds.

**Limitations of the Study**

The study found more cells related to wound healing and collagen in the silver sulfadiazine treatment group, suggesting a positive response in wound healing. However, cell viability was not assessed, and the cytotoxic nature of silver at certain concentrations could mean the observed cells may not be viable. While silver's main role in wound care is antimicrobial, supporting the prevention of surgical

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infections, its effectiveness in preventing microbial growth was not further explored. The study suggests that the appropriate dose may have been used, as seen in the higher cell count and smaller wound size, but further dose formulation is needed for human application, considering preparation type and administration method.

## CONCLUSION

This study showed that surgical wound infection modeling study in Sprague Dawley rats had higher mean number of fibroblasts, tissue neutrophils, macrophages, and collagen accompanied by a significantly narrower mean wound size in subjects treated with silver sulfadiazine compared to subjects treated with gentamicin and subjects treated with 0.9% NaCl alone. Both the number of cells and wound size differed significantly between the treatment groups with negative and positive controls, indicating the potential of silver sulfadiazine to prevent surgical wound infections and accelerate wound healing. Further research is needed for the application of silver sulfadiazine in humans to prevent surgical wounds, and this study can be a basis for future development.

## ETHICAL APPROVAL

This study received ethical approval from Medical Research Ethics Committee of Faculty of Medicine, Diponegoro University with No.105/EC-H/KEPK/FK-UNDIP/X/2024.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conceptualization, AR, BPB, EM; methodology, AR; software, AR; validation, BPB, EM; formal analysis, AR; investigation, AR; resources, BPB, EM; data curation, BPB, EM; writing—original draft preparation, AR; writing—review and editing, AR, BPB, EM; visualization, AR, BPB, EM; supervision, BPB, EM; project administration, EM; funding acquisition, AR.

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