



## Histopathological Study of Topical Effect of Doxycycline versus Fusidic Acid Treated Streptococcus-Induced Dermatitis in Mice: A randomized study

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### ARTICLE INFO.

#### Article history:

-Received:

-Accepted:

-Available online:

#### Keywords:

Awassi doxycycline,  
fusidic acid, streptococcus  
pyogen, skin

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### ABSTRACT

**S**treptococcus bacteria, particularly *Streptococcus pyogenes* (Group A *Streptococcus*), are significant pathogens responsible for a variety of skin infections, including impetigo, cellulitis, erysipelas, and more severe conditions like necrotizing fasciitis and streptococcal toxic shock syndrome. These infections often present with symptoms such as inflammation, redness, and irritation of the skin, collectively known as dermatitis. which degrade host tissues and evade the immune system. doxycycline, a broad-spectrum antibiotic, is also used due to its ability to inhibit bacterial protein synthesis, thereby halting bacterial growth and multiplication. Fusidic acid, another antibiotic, is particularly effective against Gram-positive bacteria like *S. pyogenes*, and it has demonstrated high sensitivity in treating these infections. Its anti-inflammatory properties further enhance its efficacy in reducing inflammation associated with skin infections. This study has been conducted in order to evaluate the therapeutic efficacy of doxycycline by compare with fusidic acid. The focus is on their roles as an antibiotic and anti-inflammatory agents in treating mice with dermatitis caused by *Streptococcus* bacteria. This study found that groups G3 (DOXY + VAZ), G5 (FUS), and G6 (DOXY + FUS) showed the most significant changes in their immune parameters, including LYMP and NEUT ratios, IL-6 levels, and CRP levels. These findings implied that these groups' therapies were quite successful in modifying their immune responses. Conclusion: Doxycycline is a potentially effective treatment for streptococcal dermatitis due to its dual roles as an antibiotic and an anti-inflammatory. Its capacity to suppress important inflammatory pathways and control pro-inflammatory cytokines sets it apart from other medications like fusidic acid.

## Introduction

Group A *Streptococcus* (GAS), scientifically known as *Streptococcus pyogenes*, is a gram-positive, beta-hemolytic bacterium that is sensitive to bacitracin and can be part of the normal flora in the throat, vagina, or skin (Sujithra *et al.*, 2022). The global epidemiology of GAS is characterized by the emergence of new clones with enhanced virulence and antimicrobial resistance, posing challenges to treatment and prevention (Brouwer *et al.*, 2023). There is evidence that people in developing countries; those with low socio-economic status; children, elders and immunocompromised people are more likely to contract GAS infection (Chisholm *et al.*, 2021). *Streptococcus pyogenes*, is a pathogenic bacterium that causes a number of skin lesions such as clinical impetigo, cellulitis, and erysipelas. The fluctuating global epidemiology of GAS is marked by the emergence of new clones with enhanced virulence and antibiotic resistance, complicating treatment and control efforts (Brouwer *et al.*, 2023). Doxycycline, a broad-spectrum antibiotic from the tetracycline class, is effective against a wide range of bacterial infections, including respiratory, urinary, skin infections, and sexually transmitted diseases, by inhibiting bacterial protein synthesis, thereby preventing bacterial growth and spread (Jodh *et al.*, 2022) (Shkodyak *et al.*, 2022). Fusidic acid (FA) is a steroidal antibiotic derived from the fungus *Fusidium coccineum*, primarily used to treat infections caused by Gram-positive bacteria (Sharma *et al.*, 2022).

## Materials and methods

| No | Medications           | Manufacture        |
|----|-----------------------|--------------------|
| 1  | Doxycycline 100mg     | Medochime cyprus   |
| 2  | Fusidic acid ointment | Leo Pharma Denmark |
| 3  | Vaseline              | UNILEVER USA       |

## Experimental design

Mice were used in this study. The numbers used were (60) sixty mice of The ranged weight between 27-30 gm, and their ages ranged

between (6-8) weeks. These animals were sourced from the National Center for Drug Control. Mice were divided into six different groups. Each group had 10 mice. This was done randomly to ensure fairness. . The Animals of Current Experiment showed in Figure (1).

## Application of Treatments:

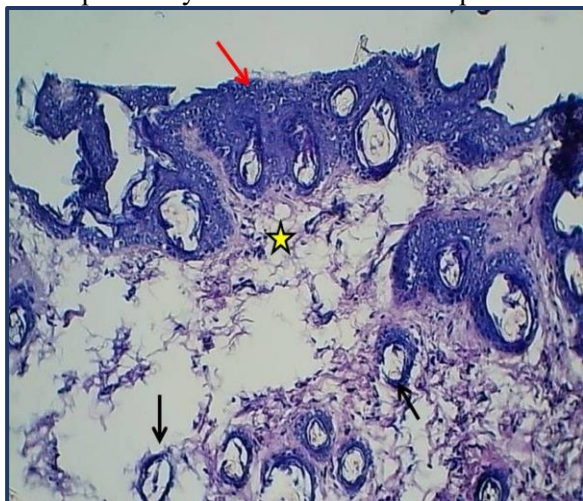
- **Negative Control Group:** No treatment was applied. The incision was left to heal naturally to observe the baseline healing process.
- **Positive Control Group:** The incision was infected with *Streptococcus pyogenes* to study the effects of infection without treatment.
- **Doxycycline with Vaseline Group:** After 7 days of infection, the wound was treated with a combination of doxycycline and Vaseline for 14 days.
- **Vaseline Group:** After 7 days of infection, the wound was treated with Vaseline alone for 14 days.
- **Fusidic Acid Group:** After 7 days of infection, the wound was treated with fusidic acid for 14 days.
- **Doxycycline with Fusidic Acid Group:** After 7 days of infection, the wound was treated with both doxycycline and fusidic acid for 14 days.



**Figure 1 :** The Animals of Current Experiment.

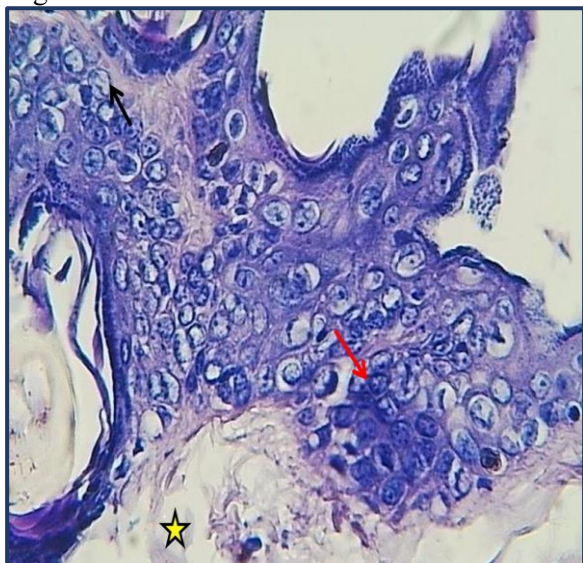
**Descriptive Histology result of skin:**

- **Control -VE Group(G1):** The results of the histopathological examination of mice skin for the control negative group sections by H&E stain.100x are represented in figure 2. which showed significant, consistent hyperkeratosis, as well as severe dermal fibrous degeneration accompanied by necrosis and tissue depletion.



**Figure 2:** section of skin (G1) shows: marked regular hyperkeratosis (Red arrow),severe dermal fibrous degeneration with necrosis (Asterisk) and tissue depletion (black arrow). H&E stain.100x.

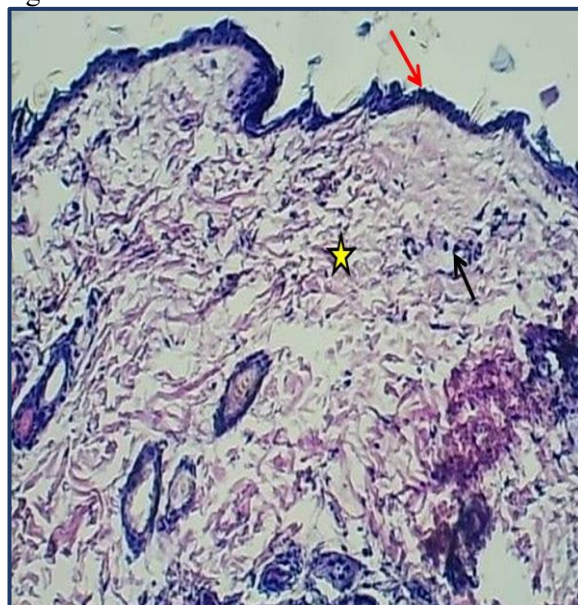
In the identical category, Figure 3 section of skin (G1) displays: prominent consistent hyperkeratosis of keratocytes accompanied by intra cellular edema, significant dermal fibrous degeneration with necrosis.



**Figure 3:** section of skin (G1) shows: marked regular hyperkeratosis of keratocytes (Red

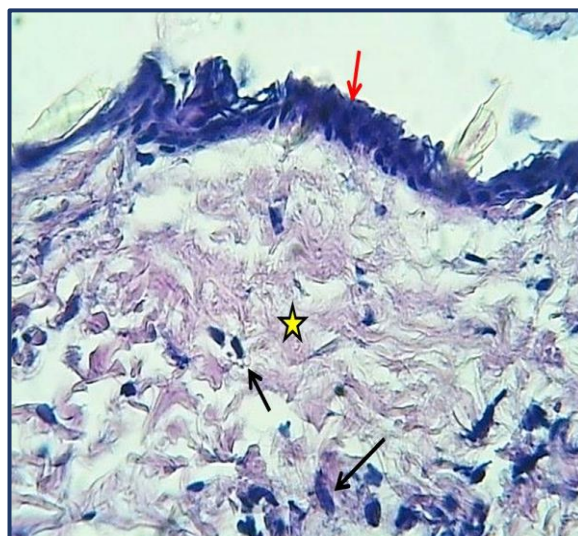
arrow) with intra cellular edema (Black arrow), severe dermal fibrous degeneration with necrosis (Asterisk). H&E stain.400x.

**Control +VE Group(G2):** Photomicrograph of mice skin of Control +VE Group showed Inadequate regeneration of the outer layer of skin, with underdevelopmentof the connective tissue and cells in the deeper layer of skin figure4.



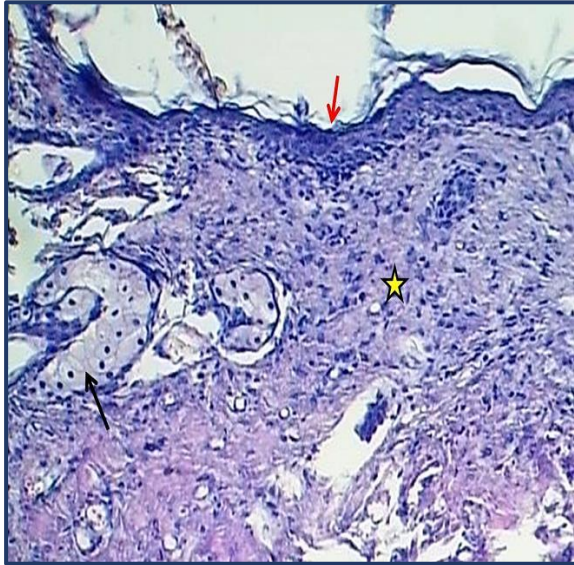
**Figure 4:** section of skin (G2) shows: poor re epithelization of epidermis (Red arrow), dermis with hypoplasia of fibrous tissue (Asterisk) and fibroblasts (black arrow). H&E stain. 100x.

In the same group figure 5 displayed a portion of skin (G2) exhibiting inadequate reepithelization of the epidermis, as well as dermis showing reduced fibrous tissue and fibroblast hypoplasia.



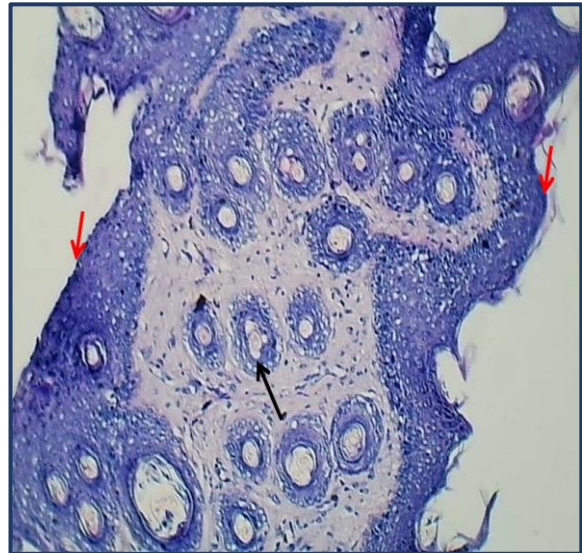
**Figure 5:** section of skin (G2) shows: poor re epithelization of epidermis (Red arrow), dermis with hypoplasia of fibrous tissue (Asterisk) and hypoplasia of fibroblasts (black arrow). H&E stain. 400x.

- **DOX+VAZ Group(G3):** Mice skin of G3 group showed the epidermal epithelium displayed typical thickness and cytoarchitecture, while the dermis exhibits regular mature fibrous connective tissue and normal sebaceous gland. as shown as figure 6



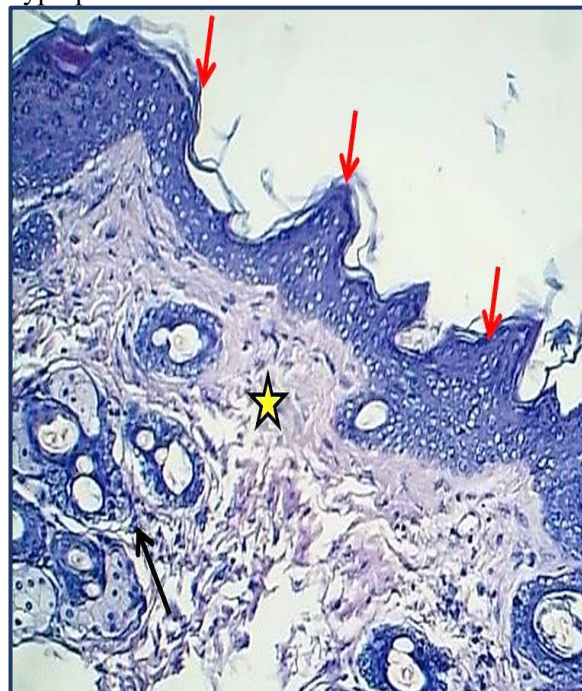
**Figure 6:** section of skin (G3) shows: normal thickness and cytoarchitecture of epidermis epithelium (Red arrow), normal mature fibrous connective tissue of dermis (asterisk) with normal sebaceous gland (Black arrows). H&E.100x.

- **VAZ Group(G4):** Mice skin of G4 group showed intense regular hyperkeratosis of the epidermis along with a mildly normal fibrous connective tissue in the dermis showing hyperplasia of hair follicles. as shown in figure.7



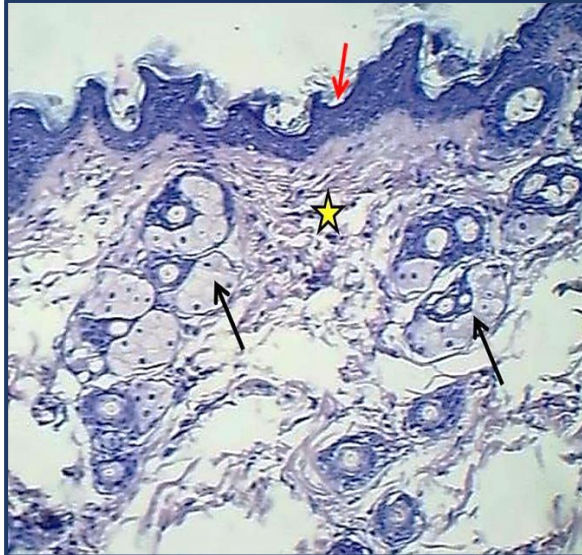
**Figure 7:** section of skin (G4) shows: severe regular hyperkeratosis of epidermis (Red arrows), slightly normal fibrous connective tissue of dermis (asterisk) with hyperplasia of hair follicles (Black arrow). H&E.100x.

Figure 8 illustrated a section of skin (G4) exhibiting pronounced regular hyperkeratosis in the epidermal cells, along with a slightly normal fibrous connective tissue in the dermis and hyperplasia of hair follicles.



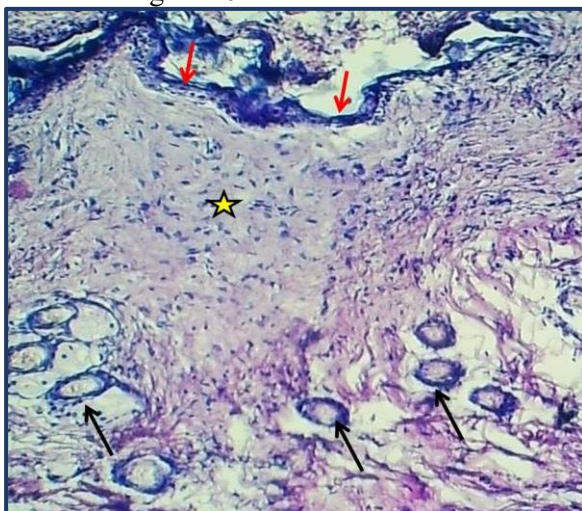
**Figure 8:** section of skin (G4) shows: severe regular hyperkeratosis of epidermal cells (Red arrows), slightly normal fibrous connective tissue of dermis ((asterisk) with hyperplasia of hair follicles (Black arrow). H&E.100x.

- **FUS Group(G5):** Mice skin of G5 group showed the epidermal epithelium displays typical thickness and cytoarchitecture, while the dermis exhibits normal mature fibrous connective tissue along with healthy sebaceous glands. As shown in figure 9.



**Figure 9 :** section of skin (G5) shows: normal thickness and cytoarchitecture of epidermis epithelium (Red arrow), normal mature fibrous connective tissue of dermis (asterisk) with normal sebaceous gland (Black arrows). H&E.100x.

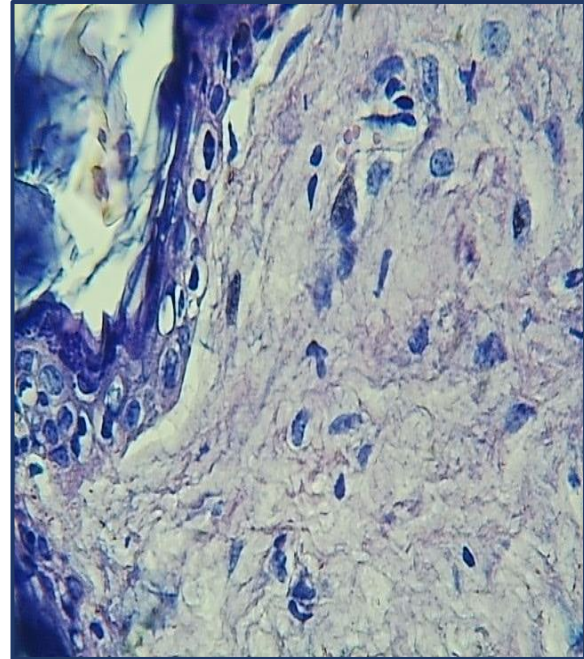
- **DOX+FUS Group(G6):**The skin section (G6) exhibits inadequate epithelization of epidermal cells, fibroplasia of mature granulation tissue in the dermis, and hyperplasia of hair follicles along with sebaceous glands. As showed in figure 10.



**Figure 10:** section of skin (G6) shows: poor epithelization of epidermal cells (Red arrows), fibroplasia of mature granulation tissue within

dermis (Asterisk) & hyperplasia of hair follicles with sebaceous glands (Black arrows) . H&E.100x.

Within the same group , figure 11 demonstrated a section of skin (G6) exhibiting inadequate epithelization of epidermal keratocytes, characterized by significant intracytoplasmic edema and fibroplasia of mature granulation tissue in the dermis.



**Figure 11:** section of skin (G6) shows: poor epithelization of epidermal keratocytes that severe intra cytoplasmic edema, fibroplasia of mature granulation tissue within dermis. H&E.400x.

## Results and Discussion

To evaluate the therapeutic efficacy of doxycycline and compare it with fusidic acid as an antibiotic and anti-inflammatory in mice with streptococcal dermatitis. Topical doxycycline may be a therapeutic option for patients with streptococcal skin infections , given its combined antibiotic and anti-inflammatory properties.

## Histopathological Assessment

The histopathological analysis of skin incisions, both with and without infection induction, reveals significant insights into the healing process and the impact of infections. In the case of incisions without infection induction (G1), the histopathological examination showed marked regular hyperkeratosis of keratocytes, indicating an abnormal thickening of the outer skin layer.

The results of the current study are consistent with Neorčić & Fejsa, 2022 observed in wound healing studies where granulation tissue formation, fibroblast migration, and neoangiogenesis are observed, leading to scar formation within three to four weeks. Also Severe dermal fibrous degeneration with necrosis and tissue depletion were noted, suggesting significant damage to the deeper skin layers. This aligned with Karppinen et al., 2019 found the typical histomorphological changes seen during the wound healing process, where irregular collagen fiber architecture and loss of skin appendages are common.

The skin incision with infection induction (G2) showed poor reepithelization of the epidermis, characterized by a thin line of proliferated keratocytes, which may indicate delayed healing. This results of present study agree with Vu et al., 2021 found Group A Streptococcus (GAS) infections, particularly those involving the Plasminogen-Associated M-Protein (PAM) and streptokinase (SK), create a localized proteolytic environment that disrupts wound architecture, leading to delayed wound retraction and impaired healing. Also The hypoplasia of fibrous tissue and the scarcity of mature collagen fibers and fibroblasts in the dermis are indicative of an impaired wound healing response, which is exacerbated by the inflammatory milieu created by the infection this agree with Papadopoulou et al., 2023 found increased expression of pro-inflammatory cytokines and delayed angiogenesis in infected wounds, which are critical for tissue repair and regeneration.

Treatment with doxycycline (G3) resulted in histopathological findings of present study showing normal thickness and cytoarchitecture of epidermal epithelial cells, indicating a positive effect on skin regeneration. The dermis displayed normal mature fibrous connective tissue with normal sebaceous gland morphology, suggesting a potential role of doxycycline in promoting tissue healing. This results agree with Moore et al., 2020 found doxycycline has been shown to reduce scar thickness and improve collagen fiber arrangement indicating its role in enhancing skin regeneration and reducing fibrosis. Also agree with Diehm et al., 2022 reported In a rodent model of capsular fibrosis, doxycycline administration resulted in increased skin

thickness and collagen density, further supporting its protective role in skin integrity and healing. In addition the study conducted by Matsui et al., 2020 found that applying doxycycline directly to the skin can help treat atopic dermatitis (AD) by reducing the development of Th2 cells, which are involved in the immune response associated with AD.

The histopathological findings of current study found treatment with vaseline (G4) led to severe regular hyperkeratosis of the epidermis, with keratocytes showing marked vacuolar cytoplasm, which may indicate an abnormal response to the treatment. The dermis exhibited slightly normal fibrous connective tissue with hyperplasia of hair follicles, suggesting a potential impact of vaseline on skin structure. This results agree with Carlsson, 2011 found Vaseline was found to have no inhibitory effects on bacterial growth and did not delay wound healing compared to untreated controls, although it did cause slight local hypergranulation. Also agree with Huang et al., 2014 indicating that while Vaseline can aid in pain management, it may not enhance the healing process.

Treatment with fusidine in (G5) resulted in histopathological findings similar to those of doxycycline treatment, with normal thickness and cytoarchitecture of epidermal epithelial cells and normal dermal fibrous connective tissue. This suggests that fusidine may also have a positive effect on skin healing and regeneration. This results agree with Liu et al., 2020 found Histopathological analysis of infected tissues treated with fusidic acid demonstrates reduced bacterial colonization and inflammation, attributed to the drug's ability to inhibit biofilm formation and  $\alpha$ -toxin production in streptococci. Also agree with Burillo et al., 2024 indicated the histopathological examination of tissues from patients with streptococcal infections treated with fusidic acid reveals a marked reduction in tissue necrosis and inflammatory markers, indicating the drug's role in mitigating the severe inflammatory response associated with these infections.

In the histopathological examination of current study found combined treatment of compound doxycycline with fusidine resulted in poor epithelization of epidermal keratocytes with severe intracytoplasmic edema, indicating a potential interaction between the two treatments. The dermis showed fibroplasia of

mature granulation tissue and marked hyperplasia of hair follicles with sebaceous glands, suggesting a complex response to the combined treatment regimen. There are no studies compare with the findings of this research.

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## Conclusions

Doxycycline is effective as both an antibiotic and an anti-inflammatory agent in treating Streptococcus-induced dermatitis in mice.

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## دراسة الامراضية النسيجية للدوكسيسايكلين الموضوعي مقابل حمض الفيوسيدك في التهاب الجلد الناجم عن العقديات في الفئران : دراسة عشوائية

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### الملخص

تعد بكتيريا العقديّة، وخاصة العقديّة القحبيّة (العقديّة من المجموعة أ)، من مسببات الأمراض الخطيرة المسؤولة عن مجموعة متنوعة من التهابات الجلد، بما في ذلك القوباء، والتهاب النسيج الخلوي، والحمرة، والحالات الأكثر شدة مثل التهاب اللقافة الناحر ومتلازمة الصدمة السامة العقديّة. غالبًا ما تظهر هذه الالتهابات بأعراض مثل الالتهاب والاحمرار وتهيج الجلد، والمعروفة مجتمعة باسم التهاب الجلد. تتضمن مسببات الأمراض لهذه الالتهابات عوامل ضراوة مختلفة، بما في ذلك بروتين M ، وكبسولة حمض الهيالورونيك، والسموم الخارجية المسببة للحمى العقديّة (SpeB) B ، والتي تحلل أنسجة المضيف وتتهرب من الجهاز المناعي. يستخدم الدوكسيسايكلين، وهو مضاد حيوي واسع الطيف، أيضًا بسبب قدرته على تثبيط تخليق البروتين البكتيري، وبالتالي وقف نمو البكتيريا وتكاثرها. حمض الفيوسيدك، وهو مضاد حيوي آخر، فعال بشكل خاص ضد البكتيريا إيجابية الجرام مثل العقديّة القحبيّة، وقد أظهر حساسية عالية في علاج هذه الالتهابات. خصائصه المضادة للالتهابات تعزز فعاليته في تقليل الالتهابات المرتبطة بعدوى الجلد.

**الكلمات المفتاحية : دوكسيسايكلين ، فيوسيدك اسيد**