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ORIGINAL ARTICLE

Protective effects of Staphylococcal Enterotoxin B (SEB) toxoid on lung and liver tissue integrity in rats during systemic infection

Dhafer Rasheed Al-Fetly[1*], Atiaf Ghanim Rhyaf[2], Hala Abbas Naji[2]

[1]*Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq*
[2]*Department of Pathology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq*

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ABSTRACT

Background and Objectives: Staphylococcal enterotoxin B (SEB), a potent superantigenic toxin produced by *Staphylococcus aureus (S. aureus)*, plays a crucial role in *S. aureus* systemic infection. This investigation sought to determine whether immunising animals with SEB toxoid could protect against an experimental acute systemic infection caused by *S. aureus.*
Materials and Methods: This study involved three groups of animals: one group was administered with SEB toxoid, and the second group was administered with intramuscular injections of normal saline, after which both were subjected to systemic *S. aureus* infection. The third group served as the negative control. After two weeks, the outcomes of the experimental systemic infection demonstrated that SEB immunisation significantly shielded organs (lung and liver) from damage in comparison to the control group.
Results: Regarding the histopathological analysis of liver and lung tissues, the control group showed minimal alterations, indicating a normal tissue state. Infected individuals exhibited severe pathology, including inflammation, necrosis, and fibrosis. The immunised group displayed a mixed profile with elevated inflammation but lower necrosis and fibrosis. Immunisation mitigated pathological changes induced by infection, fostering a more controlled response.
Conclusion: SEB plays an important role in *S. aureus* pathogenesis and immunisation, and this toxoid might protect against fatal infections of *S. aureus.*

Keywords: SEB; Methicillin-resistant *Staphylococcus aureus*; Pathogenesis; Immunization; Tissue damage; Experimental infection

INTRODUCTION

Staphylococcus aureus is a versatile pathogen inducing a wide range of diseases in animals and humans, ranging from mild to severe infections such as toxic shock (1). The bacterium comprises a set of exotoxins that play a critical role in its pathogenicity and inducing infections (2). SEB is one of the potent exotoxins produced by many Staphylococcus strains, which is responsible for toxic shock in humans, principally caused by its capacity to disrupt the cellular immune system's induction (3-5). Additionally, this toxin is classified as a superantigen, and its ability to stimulate up to 30% of lymphocytes is well-documented (4, 6). Additionally, the toxin initiates the immune response by activating toll-like receptors, which are responsible for recognising and managing the immune response against microorganisms (2,

*Corresponding author: Dhafer Rasheed Al-Fetly, M.Sc, Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq. Tel: +964-7803259734 Email: dhafer.hahmeed@qu.edu.iq

7-9). SEB binds directly to MHC II and causes harmful effects on the immune cells (7).

A recent study demonstrated that SEB toxin contributes to systemic infection caused by *S. aureus* in an animal model. The role of SEB in pathogenesis was tested by creating SEB mutants and assessing their role in comparison with the parent cell. The previous study demonstrated that systemic infection caused by *S. aureus* depends on the presence of the SEB gene, whereas cytokine expression, including IFN-γ levels, is related to the SEB toxin (7, 10, 11). Whole or subunit SEB antigens were previously used as experimental vaccines against *S. aureus*, particularly the methicillin-resistant strain. Immunisation of rats with the SEB vaccine protects the experimental animal from infection by methicillin-resistant *Staphylococcus aureus* (MRSA) (12).

Previous studies reported that immunisation with attenuated SEB induces full protection against MRSA infection in rats (13, 14). Zhao and his colleague found that the SEB multiple B cell epitope vaccine is more effective than the whole SEB antigen in clearing bacteria from rats infected with MRSA (14, 15). The research employed histopathological techniques to assess whether SEB toxoid could effectively inhibit lung and liver tissue damage caused by MRSA infection. This research could have potentially significant implications for the advancement of preventive measures and vaccines. The current study aimed to protect the activity of SEB toxoid against staphylococcus infection.

MATERIALS AND METHODS

Animal models. Fifteen healthy adult albino rats (1-month-old and weighing 200-250 g) were obtained from the animal house affiliated with our scientific institution. All rats were fed ad libitum and grouped (n=5 per cage) in standard plastic cages in an air-conditioned room with a temperature set at 25 ± 2°C. Before experimentation, the animals were allowed to acclimate for 7 days before inoculation at a 12 h light/dark cycle.

Study groups and treatment of animals. All rats were randomised into three groups of 5 rats each. The rats were randomly divided into three groups: The first group was immunised with SEB and then given an injection of *S. aureus* to evaluate the effectiveness of the SEB immunisation. The second group was the *S. aureus* infection-positive control group, and the third group served as the negative control group. Rats in the SEB test group received 100 µg SEB toxoid intramuscularly (I/M), while rats in the control groups received 50 µL PBS intramuscularly (I/M). Two weeks after the single dose immunisation, rats in the SEB test group and the infection-positive group were infected with *S. aureus* strain USA300 (BEI Resources, NIAID, NIH, NR-46070) (10^7 CFU) via intravenous tail vein injection.

Toxoid and bacterial strains. SEB was obtained through BEI Resources, NIAID, and NIH: Staphylococcal Enterotoxin B Toxoid, chemically inactivated from *S. aureus* subsp. *aureus*, NR-44235. SEB toxoid was provided in the proportion of 0.5 mg per ml. The toxoids were diluted with PBS and then injected into the tested animals (single dose at a rate of 100 µg per animal). *S. aureus* strain USA300 was obtained through the Biodefense and Emerging Infections Research Resources Repository (BEI Resources, NR-46070). A single colony of the strain was grown in tryptone soya broth for 16h at 37°C. The bacteria suspension was washed and resuspended with PBS and then adjusted to 10^7 CFU before being injected into the tested animal.

Humoral response. ELISA test was used to evaluate humoral interaction. ELISA was particularly performed to determine the concentration of Rat IgG (Immunoglobulin G). Eliza Kits (Elk Biotech, China), and the assay was performed according to manufacturer's instructions.

Histopathology and immunohistochemical assay. Rats were euthanized after 7 days of the infection using a high dose of anaesthesia. Their internal organs (lung and liver) were extracted to identify histological alterations and immunohistochemistry assays. Also, spleen was used in assay. Tissues were fixed in 10% neutral-buffered formalin before being dehydrated using a series of ethanols, followed by infiltration. Subsequently, they were embedded in paraffin (Merk®). Paraffin-embedded samples were sectioned to a 5-µm thickness, deparaffinised, rehydrated, and stained with haematoxylin and eosin (H&E) (MilliporeSigma®). All H&E-stained sections were examined using an optical microscope (Olympus, Japan). All DAB-treated (Thermo Fisher Scientific) tissue sections were

stained with hematoxylin before being examined under a light microscope (Olympus Japan) (16).

RESULTS

Humoral response. The serum samples from immunised rats were compared with the control group and analysed using ELISA to determine the level of total IgG. Immunised rats had a significantly higher mean total IgG titter (110 ± 7.91) compared to the non-immunised group (85 ± 5.52) (p-value of 0.05).

The protective effect of SEB against experiential staphylococcus infections. The recorded clinical signs were observed in the immunised and infected groups, as depicted in Table 1.

The ability of SEB toxoid to protect organs (liver and lung) during acute systemic infection was estimated by pre-immunising Wister rats with the toxoid and then infecting them with *S. aureus.*

Histological examination. The results of the histological examination of the control group, which was not infected with the bacterium or pre-immunised with the toxin, showed normal hepatic architecture. The hepatocytes contained centrally located, prominent nuclei (Fig. 1).

Animals of group one were pre-immunised with SEB toxoid and then infected. The results of the histological examination for this group showed intact liver parenchyma, severe swelling of hepatocytes, and congestion and proliferation of Kupffer cells (Fig. 2).

In group 2, which was experimentally infected with *S. aureus*, the liver showed severe hydropic degeneration, necrosis, congestion, extravasated RBCs, infiltration of inflammatory cells, and loss of radial arrangement of hepatic cords around the central vein, as demonstrated in Fig. 3.

The lungs showed normal alveoli & normal alveolar septa in the control group (Fig. 4).

In group one, the lungs were characterised by destroyed alveoli (emphysema), thrombus, and thickening of interstitial tissue. There was also mild infiltration of inflammatory cells in interstitial tissue with mild desquamation of epithelial cells lining bronchioles. Moderate pulmonary emphysema was also observed (Fig. 5 A and B). In group 2, the lungs showed severe interstitial pneumonia, haemorrhage, and thrombus (Fig. 5 C and D).

Immunohistochemical analysis. Immunohistochemical analysis showed moderately stained cells in the liver of the immunised group (Fig. 6 A and B) compared with group 2, represented by strongly stained cells in the liver (Fig. 6 C and D).

Similar to the liver, immunohistochemical analysis showed moderately stained cells in the spleen and lung of the immunised group (Fig.7 A and C) compared with group two, which was represented by strongly stained cells in the spleen and lung as shown in Fig. 7 B and D.

Regarding the histopathological scoring index, Table 2 offers a glimpse into the impact of *S. aureus* infection and SEB immunisation on histopathological alterations in the liver and lung of rats. The data suggests that *S. aureus* infection is linked to elevated scores in

Table 1. Comparative clinical signs in rats of *S. aureus* infected group and SEB Immunised group.

Clinical signs	Groups	
	Infected	Immunised
Fever	Severe	Moderate to mild
Lethargy	Severe	Mild reduction in activity levels and energy
Change of appetite	Anorexia	Slight loss of appetite
Weight Loss	Decreased body weight compared to baseline measurements	Minimal decrease in body weight compared to the infected group
Respiratory Signs	Difficulty breathing, increased respiratory rate	Less respiratory distress, relatively normal respiratory rate
Abscess Formation	Localized collection of pus within tissues, often accompanied by swelling and inflammation	Smaller abscesses or fewer instances of abscess formation
Purulent Discharge	Presence of pus and thick discharge from the infected site	Smaller amount or absence of purulent

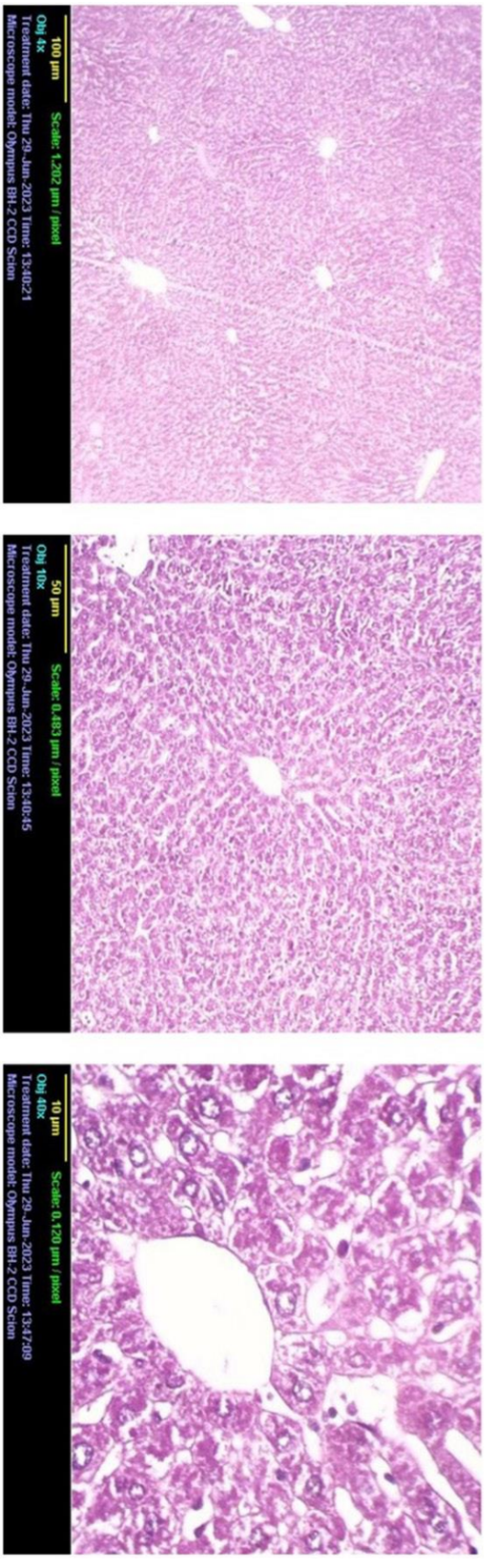

Fig. 1. Section of the liver showed normal histology. The hepatocytes radiated evenly from a central vein and contained centrally located prominent nuclei. H&E, 4X, 10X, and 40X.

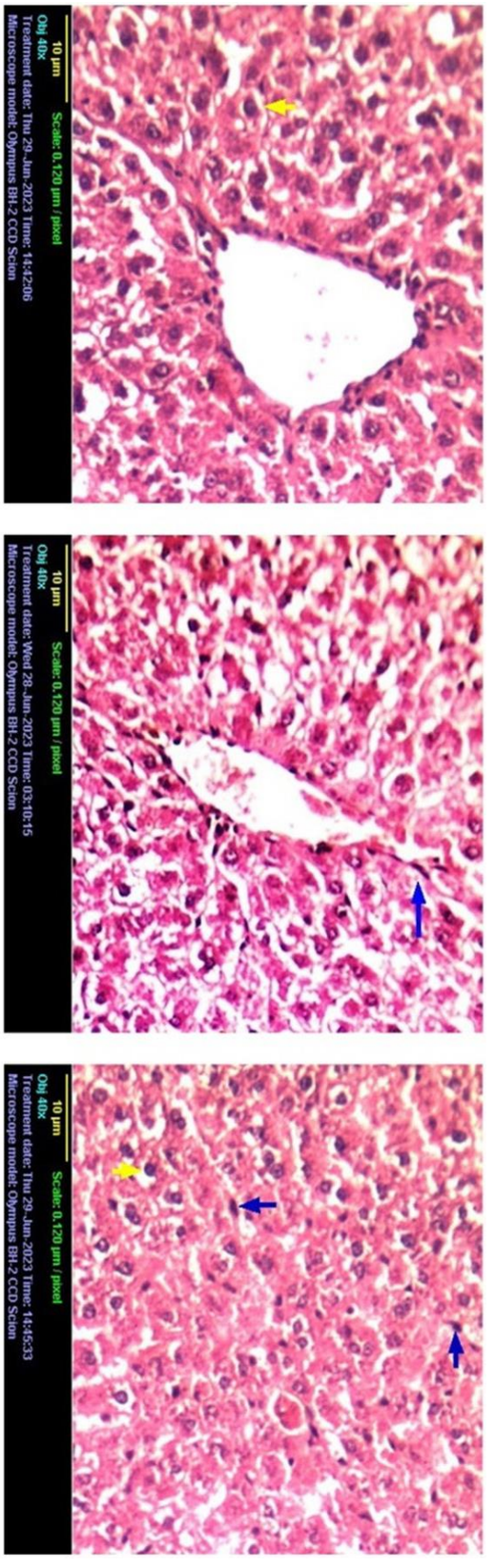

Fig. 2. Section of the liver showed severe swelling of hepatocytes (yellow arrows) and congestion and proliferation of Kupffer cells (blue arrows). H&E, 40X.

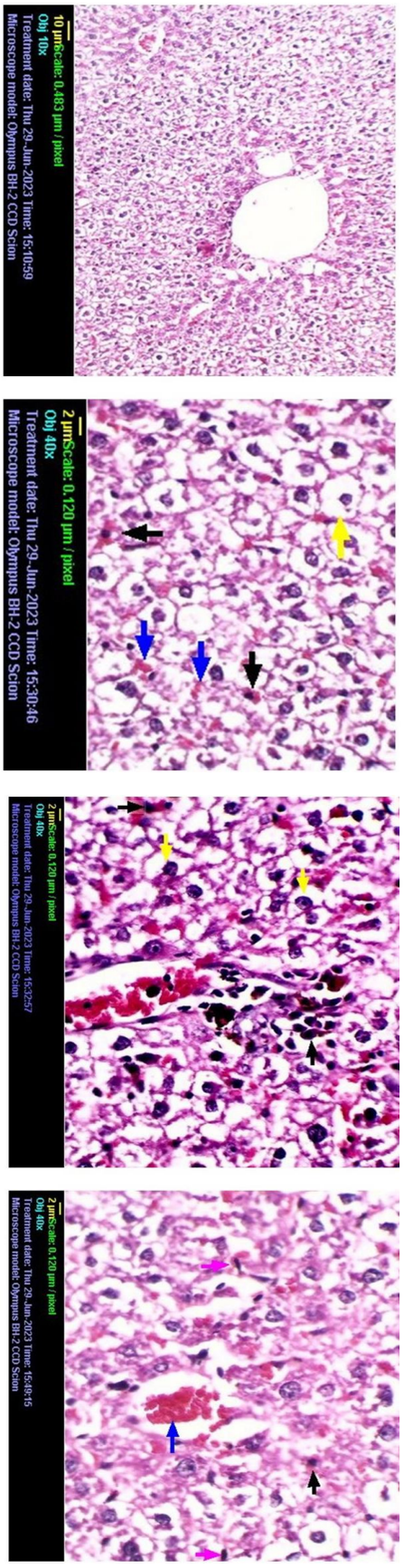

Fig. 3. Section of the liver showed severe hydropic degeneration (yellow arrows), necrosis, congestion and extravasated RBCs (blue arrows), infiltration of inflammatory cells (black arrows), the proliferation of Kupffer cells (pink arrows), and loss of radial arrangement of hepatic cords around the central vein. H&E, 10X and 40X.

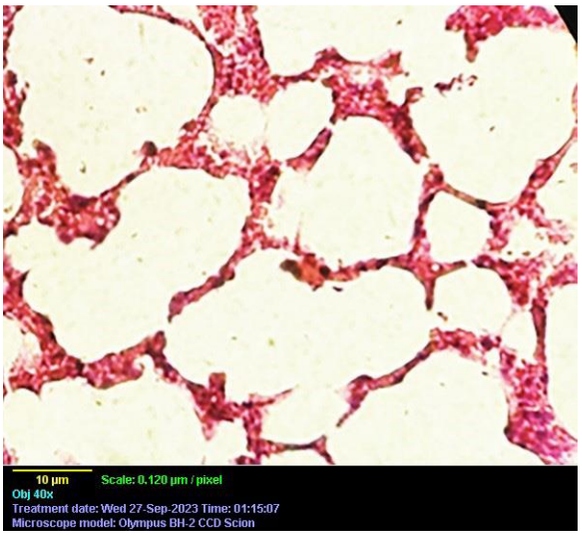

Fig. 4. Section of the lung showed normal lung alveoli & normal alveolar septa in the control group.

necrosis, fibrosis, and oedema when compared to the control group. Conversely, SEB immunisation appears to affect specific parameters differently, as evidenced by the scores for granuloma and regeneration.

As indicated in Table 3, the severity of the illness and the recorded damage to the liver and lungs had a substantial impact on the animals' survival rates. This table presented an in-depth summary of the impact of *S. aureus* infection and SEB immunisation on their survival. It shows that infection resulted in a lower survival percentage (60%) than the control and immunised groups, which had a 100% survival rate.

Our results demonstrate that immunising animals with SEB toxoid protect the rats from damage during systemic infection.

DISCUSSION

The previous lack of evidence showcasing SEB's capacity to induce systemic immune activation after exposure via nonenteric mucous membranes, particularly the nasal tract, is noteworthy. This holds significant clinical relevance because the nasal passage is a frequent location for staphylococcal colonisation (6). *S. aureus* produces a range of toxins that affect the bacterium's pathogenicity (17-19). Among these toxins, SEB is traditionally associated with food poisoning. Nevertheless, recent investigations have revealed that SEB also impacts *S. aureus*-induced skin and systemic infections significantly, as documented

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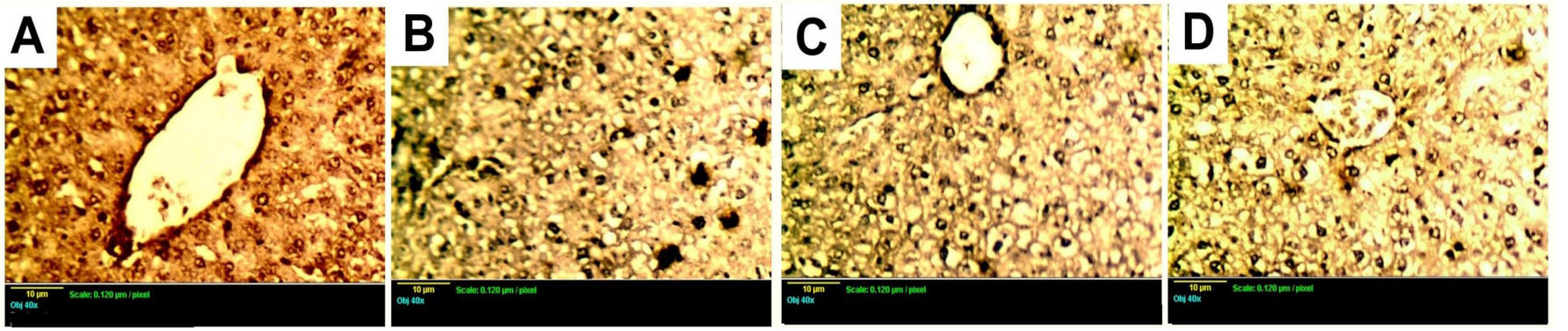

Fig. 6. Immunohistochemical analysis of the liver showed moderately stained cells in the liver of the immunized group (A&B) compared with the infected, which was represented by strongly stained cells in the liver (C&D). 40X

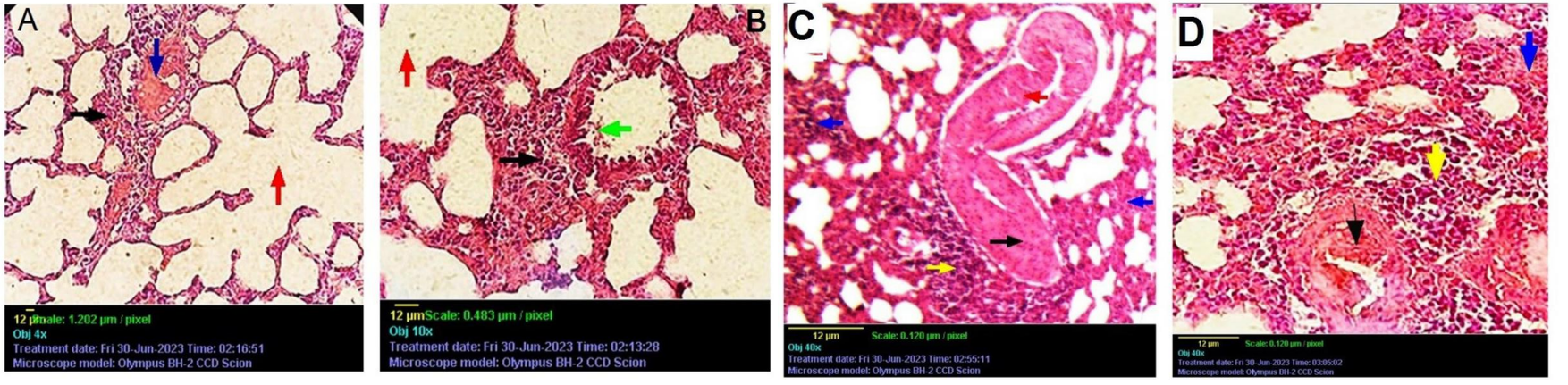

Fig. 5. A and B Section of the lung showed destroyed alveoli (emphysema) (red arrow), thrombus (blue arrow), thickening of interstitial tissue (black arrow) and mild desquamation of epithelial cells lining bronchioles (green arrow). H&E, 4X and 10X. The C and D Lung sections showed severe hyperplasia of the bronchial wall (black arrows) and obstruction of the bronchial lumen (red arrows), thickening of the alveolar wall (blue arrows), and infiltration of the inflammatory cells (yellow arrows). H&E, 40X.

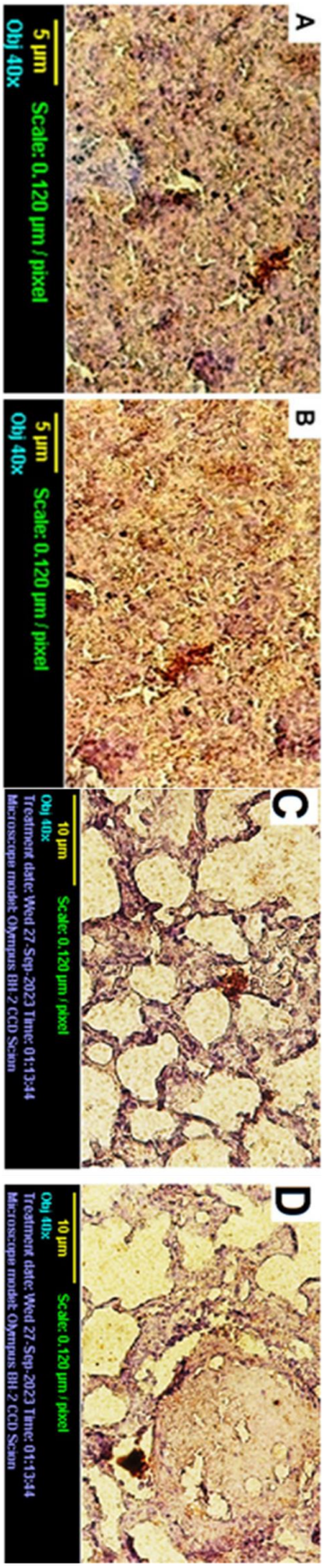

Fig. 7. Immunohistochemical analysis of the spleen showed moderately stained cells in the spleen of the immunised group (A) compared with group two, represented by strongly stained cells in the spleen (B). Immunohistochemistry stain on lung tissue in the immunised group demonstrated cells with moderate staining in the lung (C). In contrast, in the infected group, the lung tissue displayed strongly stained cells (D).

in recent studies (20, 21).

In a previous study, mutant SEBs were generated in *S. aureus* ST59, and their virulence was assessed using a mouse infection model. The results of the study demonstrated that SEB has a critical contribution to staphylococcus systemic infection (2, 22). Targeting SEB via drug or vaccine is a promising strategy to prevent lethal *S. aureus* infection (23, 24). Several studies have demonstrated that SEB is immunogenic and can strongly induce the immune system of a particular lymphocyte (4, 7, 15). Furthermore, SEB immunisation can protect rats against MRSA infection (14). However, no previous studies have been performed on the pathological aspect related to immunisation with SEB; therefore, the current study was conducted to assess the protective activity of the SEB vaccine against tissue damage during systemic infection of MRSA.

Our results demonstrated that immunisation with chemically inactivated SEB toxin results in the full protection of the lungs and liver after being infected with MRSA in comparison with non-immunised rats. This finding partially agreed with Bae's findings (2), which showed that SEB contributes to systemic infection of *S. aureus.* However, more clinical trial studies are required to prove the effectiveness of this type of vaccine against fatal systemic infection caused by *Staphylococcus* strains.

Unlike our present investigation, previous histopathological studies did not identify notable lung alterations. This discrepancy might be attributed to the relatively brief duration compared to our study, which might not have allowed for a comprehensive examination of histopathological changes. Consequently, the author suggested a more extended temporal study to thoroughly investigate histological changes correlated with cytokine levels (25-29). Further studies are needed to prove the efficacy and safety of SEB toxoid as a vaccine candidate against staphylococcal infection.

CONCLUSION

Overall, these findings underscore the potential efficacy of immunisation in modulating the impact of infection on liver and lung tissues. This study suggested that SEB toxoid can actively protect against systemic infection caused by *S. aureus* in rats. SEB

Table 2. Comparative histopathological assessment of liver and lung changes in rats: *S. aureus* infection vs. SEB immunisation across three groups (control, infected, and vaccinated).

Histopathological changes	Groups					
	Control		Infected		Immunised	
	Liver	Lung	Liver	Lung	Liver	Lung
Inflammation	1	0	4	3	1	1
Necrosis	0	0	4	3	3	2
Fibrosis	0	0	5	2	1	0
Granuloma	0	0	3	2	4	4
Regeneration	0	0	2	2	2	2
Infiltration	1	0	4	4	1	1
Edema	1	0	3	5	1	2
Hemorrhage	0	0	2	3	2	2

Table 3. Rat survival rates following experimental *S. aureus* infection and SEB vaccination in control, infected, and vaccinated groups.

Group	Number of Rats	Surviving Rats	Survival Percentage
Control	5	5	100%
Infected	5	3	60%
Immunised	5	5	100%

plays an important role in *S. aureus* pathogenesis and immunisation, and this toxoid can effectively protect against fatal infections caused by methicillin-resistant *S. aureus.*

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