

## Skin response to lipopolysaccharide-induced sepsis based on histology



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### ABSTRACT

**Introduction:** The skin is the largest organ of the human body. Sepsis is a serious disease and causes multi-organ damage, with a high cause of death. However, the mechanism by which sepsis can damage the skin structure is not clear. This study aimed to investigate skin damage in a lipopolysaccharide-induced endotoxemia (LPS) model widely used in mice.

**Methods:** This study used an experimental design with a control group that was randomized solely for the post-test. White mice (*Mus musculus*), the study's population, made up this group. LPS injection for 4 hours (LPS+4h, n=4), LPS injection for 8 hours (LPS+8h, n=4), and control group (Ctrl group, n=4) were randomly assigned to mice. The SPSS ver. 25 statistical analysis program was used. One-Way ANOVA was used to compare more than three sets of data, and Tukey's multiple comparison test was used to assess the results. The p-value of 0.05 was used to determine if the difference was significant.

**Results:** Based on this study, LPS injection increased the leukocyte concentration significantly (p-value<0.05) in the 4 h and 8 h vs control group. LPS-induced sepsis decreased body weight significantly (p-value<0.05). The morphology of skin thickness in the control group was normal, according to the results of the histopathologic study of the area stained with hematoxylin and eosin. However, the thickness was decreased in mice after 4 hours and 8 injections of LPS significantly (p-value<0.05).

**Conclusion:** LPS-induced septic mice cause damage to the skin, and changes in skin thickness due to the inflammatory process due to sepsis.

**Keywords:** Sepsis, LPS, skin, thickness.

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### INTRODUCTION

The skin is the biggest organ in the human body and is largely responsible for preserving homeostasis and shielding the body from the harmful effects of the outside world. The skin not only performs essential tasks but also plays a significant element in the immune system's fight against infections.<sup>1</sup> Skin-resident cells such as Langerhans cells, keratinocytes, melanocytes, mast cells, and macrophages emit tiny, hormone-like signal peptides known as cytokines that serve as local immunity modulators or draw in more immune cells in response to internal or external inputs.<sup>2</sup> Specific membrane receptors that are present in the majority of cells are required for their action. Even though a number of skin conditions, such as psoriasis, atopic dermatitis, seborrheic dermatitis, and contact dermatitis, are characterized by inflammation, their cellular immune responses and cytokine

profiles differ from one another.<sup>3</sup> Some in vivo as well as in vitro models replicate the skin's inflammatory response.<sup>4,5</sup>

Neutrophils and monocytes move quickly into the damaged skin during the inflammatory phase, which primarily involves activation of the innate immune system. Hemostasis occurs concurrently with this phase, which is referred to as the first stage of wound healing.<sup>6</sup> There is several studies that explained the mechanism of inflammation in the skin, the free radical theory is the one of mechanism that is popular in the sciences.<sup>7</sup> According to the free radical hypothesis, improperly produced reactive oxidative species (ROS) can cause DNA damage as well as oxidative proteins, nucleoid acids, and lipids.<sup>8</sup> On the other hand second theory is the programmed cellular senescence theory.<sup>9</sup> Keratinocytes, macrophages, dendritic cells, and mast cells are among the local skin cells that are exposed to danger

signals as a result of injury. These warning signs may be roughly divided into two categories: PAMPs are pathogen-specific compounds, such as polynucleotides and essential polysaccharides produced by bacteria, that are not present in the host. Stressed cells going through necrosis emit chemicals known as damage-associated molecular patterns (DAMPs). Bacterial metabolites, notably short-chain fatty acids generated by anaerobic bacteria, impaired the function of white blood cells. More tissue damage is caused by higher levels of cytotoxic enzymes and ROS generation. Exotoxins from bacteria attack different cell types and cause tissue necrosis. This situation is made worse by local hypoxemia brought on by vascular blockage.<sup>8,10</sup> However, the histological of skin damage after sepsis in the skin is still unclear. This study examined the skin damage after LPS induction in a short time.

## METHODS

### Study Design

This study used an experimental design with a control group that was randomized solely for the post-test. In this investigation, male mice *Mus musculus* aged 8 weeks were employed. The vivarium chamber was kept at a regulated temperature of 22.5°C and was kept on a 12-hour light/dark cycle (lights on at 9 am, lights out at 9 pm). Before starting the experimental treatment, all rats were confined for 7 days to acclimate. The three groups of mice were arbitrarily divided into the Control group (Ctrl group, n=4), LPS+4h (LPS+4h, n=4), and LPS+8h (LPS+8h, n=4). Sigma-Aldrich Company (Sigma-Aldrich Co., St. Louis, MO, USA) acquired LPS (*Escherichia coli*; O127:B8). LPS was injected intraperitoneally at a dose of 25 mg/Kg.

### Data collection

The abdomen skin was immersed in paraffin, fixed by immersion in 10% buffered formaldehyde overnight, and then cut into coronal slices that were 5 m thick. Hematoxylin and eosin was used to stain brain slices after deparaffinization for standard histological analysis.

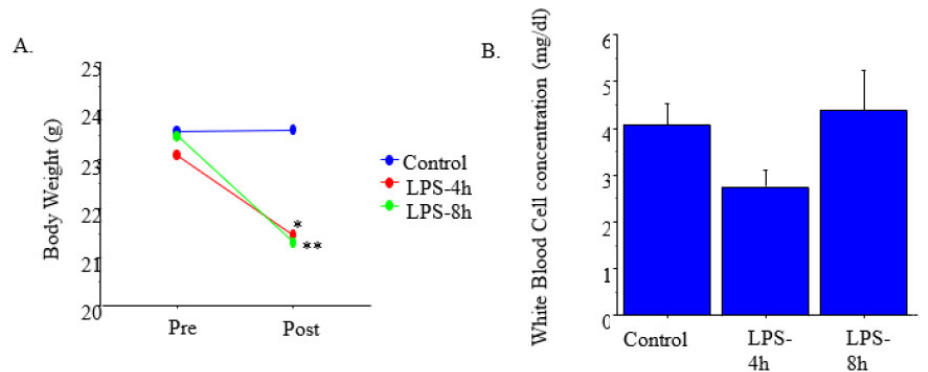
### Data analysis

The mean and SEM of the data are displayed. The SPSS ver. 25 statistical analysis program was used. One-Way ANOVA was used to compare more than three sets of data, and Tukey's multiple comparison test was used to assess the results. When the p-value was 0.05 or below, the difference was deemed significant.

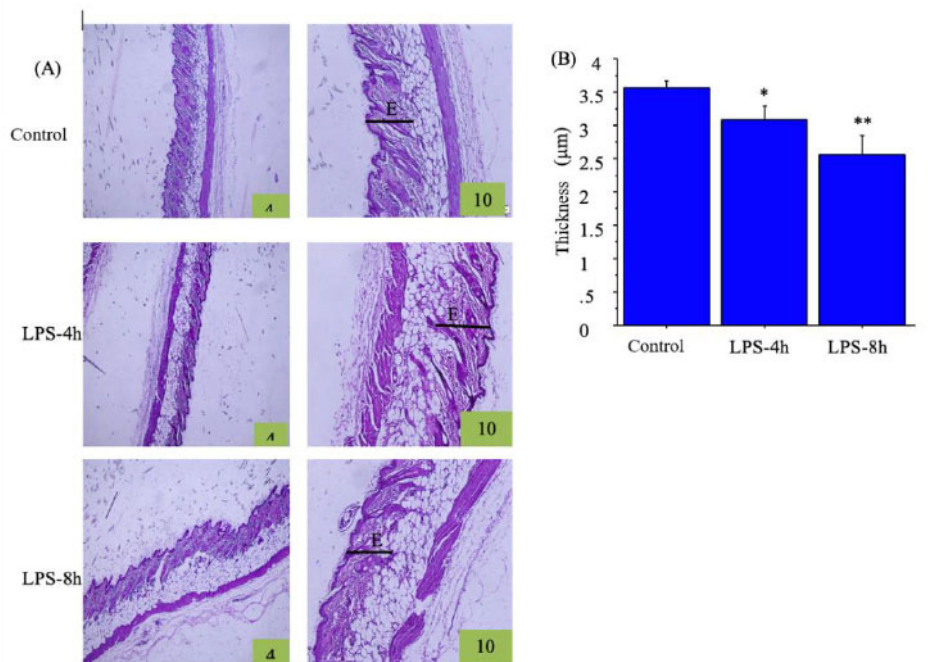
## RESULTS

### Leukocyte concentration and body weight changes following LPS-induced sepsis

White blood cells were examined to assess how an LPS injection caused sepsis. Figure 1A demonstrated that, when compared to a control group, the 4 h and 8 h groups of LPS injection considerably (p-value<0.05) increased the blood's leukocyte concentration. On the other hand, LP-induced sepsis substantially reduced bodyweight (p-value<0.05). (Figure 1)



**Figure 1.** After LPS induction, white blood cell concentration decreased in 4-h and then increased in 8-h. Leukocyte concentration and body weight changes following (A) Weight loss in the LPS group. The mean of the data from 8 animals in each group and SEM are represented as vertical lines. \*p-value<0.05, \*\*p-value<0.01 in comparison to control.



**Figure 2.** Representative top skin specimen histological cross-sections. (A) Histological slice (B) Histology-based measurements of skin thickness. (n = 8 in each group). \*; p-value<0.05, \*\*; p-value<0.01, and all values are mean ± SEM.

### Skin Histopathologic Alterations Following LPS Induced Sepsis

The morphology of skin thickness in the control group was normal, according to the results of the histopathologic study of the slice stained with hematoxylin and eosin. The thickness did, however, significantly diminish in mice after 4 hours and 8 injections of LPS (p-value<0.05).

## DISCUSSION

Infection combined with systemic symptoms of infection is known as

sepsis.<sup>11</sup> As a result, it is more than just the local organ pathological damage and suggests that one or more other crucial organs may not be functioning properly. Our study's major objective was to look at skin thickness in LPS-induced sepsis. In previous investigations, it was discovered that sepsis disturbs the tissue that connects wounds.<sup>12</sup> However, another study found that septic patients had delayed epidermal wound healing. This finding suggested that sepsis had an enhanced blood flow response, which may have resulted from a

high level of systemic inflammation.<sup>13</sup>

According to a previous study, in this study showed that LPS-induced sepsis in mice model was decreased the skin thickness. This finding is related to mechanism of inflammation in the sepsis mechanism. Recent research has clarified the signaling cascade downstream of interleukin and TNF receptors, two receptors linked to sepsis. In addition to LPS-induced inflammatory systems, this model can be used to monitor and analyze several aspects of cutaneous disorders, oxidative stress, skin irritation, and functional tests.<sup>14</sup> Based on study by Sommer et al., 2013 stated that the serum levels of IL-6 and TNF- $\alpha$  in septic and non-septic mice to confirm sepsis following the CLP procedure. The weight of the spleen was assessed for persistent inflammatory reaction. After sepsis was induced, septic animals had significantly heavier spleens than non-septic mice which were both statistically significant.<sup>12</sup>

Weight of the spleen was also assessed for persistent inflammatory reaction. After sepsis was induced, septic animals had significantly heavier spleens on days 10 and 16 (0.55 g and 0.35 g, respectively) than non-septic mice (0.21 g and 0.17 g, respectively), which were both statistically significant ( $p < 0.001$  for days 10 and 16).

The secreted cytokines have an important role as a modulator of the innate immune system and maintain the homeostasis and function of the various types of cells that make up the skin. Where this is related to the signaling pathway mediated by certain receptors through the activation of JAK-STAT and NF- $\kappa$ B transduction signals in inflammatory reactions in the skin.<sup>15</sup>

We assessed the inflammatory response to LPS stimulation in the current investigation. The quantity of leukocytes in the blood was observed to have decreased. Leukocyte production has increased, indicating the presence of an infectious process that led to the induction of sepsis by LPS. Similar to this outcome, the earlier study looked at the significance of septic pathophysiology's temporal responses at various stages.<sup>16</sup> After 3 hours of LPS treatment in rats, a related investigation discovered that the WBC count in whole blood drastically dropped.<sup>17</sup> There are still

many limitations to this study, including the lack of any design- or analysis-based adjustments and any further compounding variables that might affect the outcomes.

## CONCLUSION

In conclusion, we demonstrated that LPS-induced septic mice cause damage to the skin, that changes in skin thickness due to the inflammatory process due to sepsis. Further studies are needed to validate and re-evaluate these findings so that these finding can be used as a base of further studies and treatment.

## FUNDING

The authors declare no funding in this study.

## CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

## ETHICAL STATEMENT

The Use Committee of Hang Tuah University gave its approval to all techniques and processes (I/032/UHT.KEPK.03/VI/2020). In accordance with the Handbook for the Care and Use of Laboratory Animals, experimental methods were carried out.

## AUTHOR CONTRIBUTION

All authors contributed equally to this study.

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