Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2095-2099 | ISSN:2251-6727

The Role of *Clitoria ternatea* Nanogel (CTNG) in Burn Wound Healing in a Rat Model

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(Received: 16 January 2026 Revised: 25 February 2026 Accepted: 17 March 2026)

KEYWORDS

Clitorea ternatea nanogel, , IL-6, fibroblast, burn, wound healing

ABSTRACT:

Introduction: Burn injuries trigger a strong inflammatory response. Continuous inflammation can delay the wound-healing process. Clitoria ternatea is known to possess potent anti-inflammatory and antioxidant activities; however, its effectiveness may be improved through nanogel formulation.

Objectives: This study aims to evaluate the effect of Clitoria ternatea nanogel (CTNG) on interleukin-6 (IL-6) levels and fibroblast counts in a rat burn wound model.

Methods: This experimental study used a post-test only control group design using 30 male Wistar rats divided into five groups: normal control, negative control K (–), K1 treated with sulfadiazine, K2 treated with 2.5% CTNG, and K3 treated with 5% CTNG. A first-degree burn was induced on the dorsal area of the rats, and topical treatment was conducted for 14 days. The evaluated parameters included tissue IL-6 levels measured by ELISA and fibroblast counts assessed using Hematoxylin–Eosin (HE) staining. Data were analyzed using one-way ANOVA followed by post hoc tests.

Results: The K (–) group shows the highest IL-6 levels and the lowest fibroblast counts. The K2 and K3 groups show lower mean IL-6 levels accompanied by higher fibroblast counts compared with the K (–) and K1 groups. The K3 group treated with 5% CTNG shows significantly lower IL-6 levels and higher fibroblast counts than the K1 and K2 treatment groups ($p < 0.05$).

Conclusions: Administration of CTNG significantly influences burn wound healing as indicated by reduced IL-6 levels and increased fibroblast numbers, with 5% CTNG identified as the most effective dose.

1. Introduction

Burn is a traumatic injury caused by skin contact with a heat source like extreme temperature, electricity, radiation, friction, or chemical substance. The severity of the burns depends on the affected body surface area, affecting patients' morbidity and mortality [1]. According to the World Health Organization (WHO), more than eleven million people get burn trauma per year, resulting in 180.000, 95% of them happened in low-income and underdeveloped countries [2].

Burn trauma undergoes a process of inflammation. proliferation, and remodeling. Inflammation is a process of releasing histamine, free radicals, and other inflammatory cytokines that trigger local reactions such as vasodilation and tissue edema. This process carries neutrophils and monocytes to the lesion site, sending chemotactic signals that recruit macrophages [3]. The inflammatory response strongly occurs in burn tissue lesions. The inflammation is marked by inflammatory cells infiltration, increased pro-inflammatory cytokines levels (TNF-α, IL-1β, IL-6), as well as several chemokines, including macrophage inflammatory protein-1α and -1β (MIP-1α, MIP-1β) and monocyte chemoattractant protein-1 (MCP-1) [4].

The healing process of the burn is influenced by neoangiogenesis, the activation of local immune response, and the existence of epidermal and fibroblast growth factors. Fibroblast acts to decrease the lesion diameter, together with new blood vessels and extracellular matrix form granulation tissue, causing keratinocytes to migrate in order to heal the skin integrity [5].

A conventional approach plays an essential role in initial burn treatment; however, the healing process takes time

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 2095-2099 | ISSN:2251-6727

[6]. With the advancement of biotechnology and biomaterial, numerous innovative and effective burn treatments emerge.

One of the potential treatments is *Clitorea ternatea* (CT). CT contains flavonoids and phenols, beneficial because of their antioxidant and anti-inflammatory activity [7]. It is proven that the extract gel of CT increases IL-10 gene expression level, preventing hyperpigmentation and boosting GPx levels in order to inhibit UV-B–induced photo-oxidative stress [8]. Another prior study found that ethanolic extract of CT at concentrations of 0.1%, 0.2%, and 0.4% is beneficial to heal incision wounds in New Zealand rats [9]. One of the useful efforts to maximize the effectiveness of drug delivery systems and wound management is nanotechnology innovation. Nanogel is a 3D polymer network covering therapeutic agents, providing controlled and sustained release [10]. Referring to the potentials CT holds, formulated in the form of nanogel, this study aims to explore the impact of CTNG for wound healing.

2. Objectives

Concerning the potentials of CT, formulated in the form of nanogel, the aim of this study is analyzing the impact of CTNG toward wound healing with IL-6 levels and fibroblast counts as parameters.

3. Methods

Laboratory animal

This experimental study employed a post-test only control group design. There were 30 males white Wistar rats from the PSPG laboratory, UGM Yogyakarta. Healthy rats aged 2-3 months old and weighed 150-200 grams were included. After a week, the rats' fur was shaved. In order to make a first-degree burn, a 2-cm iron plate, heated in boiling water for 5 minutes, was applied to the back for 10 seconds per group.

Experimental design

The rats were randomly assigned into five groups, each consisting of six animals: KN (healthy control group), K (–) (burn-injured rats treated with placebo gel), K1 (burn-injured rats treated with the standard drug sulfadiazine), K2 (burn-injured rats treated with 2.5% nanogel), and K3 (burn-injured rats treated with 5% nanogel). On day 15, skin samples were collected, followed by tissue processing and histological examination.

Measurement parameter

The specimen observation was conducted at the Burn Academy Laboratory located in Semarang. The sample, fixed in BNF solution, was placed into the tissue basket and was labeled. The tissue block, sectioned using a microtome at a thickness of 5 µm, was placed in a glass slide coated with Entellan adhesive. The specimen was stained using *Hemato Eoxiclin* (HE). The fibroblast was measured through ten observations, from which the average number of fibroblasts was found.

IL-6 levels were measured at the PSPG Laboratory, Gadjah Mada University (UGM), Yogyakarta. The levels were analyzed using tissue samples by the ELISA method. Meanwhile, the measurement of the absorbance was conducted using a spectrophotometer at a wavelength of 450 nm.

CTNG preparation

Clitorea ternatea was pulverized through a process called maceration using 96% ethanol. 200 mg of gel base with 2.5% and 5% butterfly pea flower extract composed the nanogel. Carbopol was first developed using boiling water before propilenglikol and nipagin were added. Constant stirring was done to make the formulation homogenous.

Data analysis

This study employed one-way ANOVA, followed by a post hoc test. Statistically, the data were significant, showing 95% reliability with a p-value of < 0.05. Data were analyzed using SPSS 26 and Graph Pad Prism 8.

Ethical clearance

This study referred to ethical clearance issued by the Medical/Health Research Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, No. 536/XII/2024/Ethics Committee.

4. Results

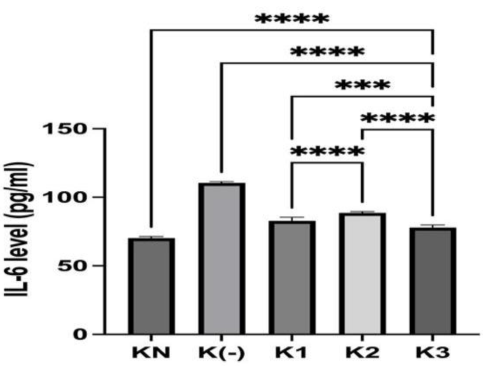

Figure 1. IL-6 Levels

Figure 1 illustrates that the average IL-6 level in K (-) is the highest. K3, the treatment group receiving 5% CTNG

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JCHR (2026) 16(2), 2095-2099 | ISSN:2251-6727

has the lowest IL-6 level, compared to the other treatment receiving SSD and 2.5% CTNG.
Compared to the normal group (p<0,05), K (-), a group consisting of the rats with burn injuries, has a significantly higher level of IL-6. Meanwhile, among the treatment groups, K1 and K2 (p<0,05), the average level of IL-6 is significantly lower.

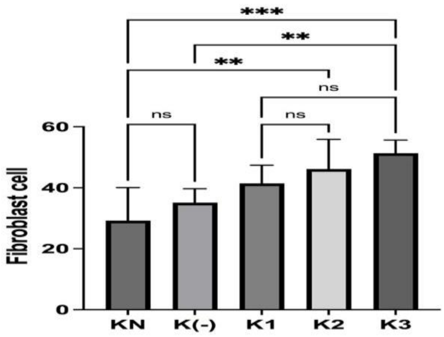

Figure 2. Number of fibroblast cells

Figure 2 presents the average number of fibroblast cells. The average number of fibroblast cells in K (-) is lower than those of K1, K2, K3. The average number of fibroblast cells in K3 is significantly higher than K (-) (p<0.05). Among the treatment groups, K3 has a higher average number of fibroblast than K1 and K2. The findings are in line with the microscopic appearance, showing that K3 has the highest average number of fibroblast cells.
The results are in line with the microscopic findings, in which the K3 group showed the highest number of fibroblasts. The dense collagen fibers are found in K3, followed by K2 and K1. Figure 3 displays the findings.

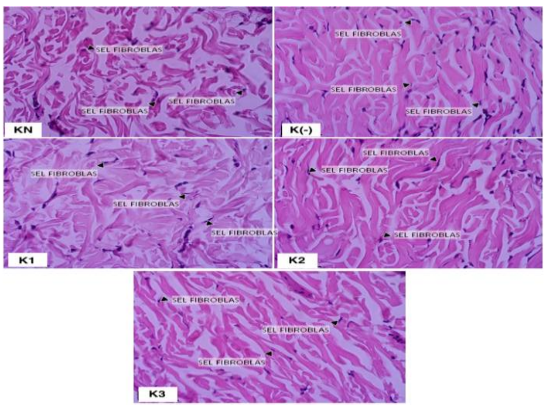

Figure 3. Histopathological Appearance with HE Staining

5. Discussion

Burn trauma involves a series of immune responses that activate the activity of specific immune cells. The progression of burn wound lesions is important for restoring immune homeostasis, which at the end enables efficient wound healing and prevents the development of complications[11]. An inflammatory response occurs when healthy tissue is damaged by a burn injury. The balance between pro-inflammatory responses and anti-inflammatory activity is therefore essential in burn wound healing. One of the key pro-inflammatory mediators is interleukin-6 (IL-6)[12]. This pro-inflammatory mediator increases during the first week following burn trauma and subsequently decreases within approximately five weeks.
Comprehensive research is required to identify new approaches for modulating host defense capacity, preventing complications arising from abnormal immune responses, and treating burn injuries more efficiently.

Effect of CTNG on IL-6 Levels in Wound Tissue
The mean IL-6 level in the negative control group K(–) is significantly the highest, indicating a strong inflammatory response induced by burn trauma. IL-6 plays a role in prolonging the inflammatory phase and contributes to secondary tissue damage when its levels remain high for an extended period. This finding is consistent with previous reports showing that IL-6 increases markedly during the early phase of burn injury and correlates with the severity of tissue inflammation.[13]
The treatment groups show significantly lower mean IL-6 levels compared with the negative control group K (–), particularly in the CTNG 5% group (K3). These results are comparable to those observed in the K1 group. This finding indicates that the CTNG formulation is capable of suppressing the inflammatory response more effectively than the standard therapy, sulfadiazine. This effect is likely due to flavonoids, anthocyanins, and phenolic compounds in *Clitoria ternatea*, which possess anti-inflammatory properties through inhibition of the NF-κB signaling pathway and reducing pro-inflammatory cytokine.[8]
In addition, the nanogel formulation enables a more stable and sustained release of active compounds within the wound tissue, thereby maintaining local anti-inflammatory effects over a longer period. This is consistent with the concept of nanogels as effective

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JCHR (2026) 16(2), 2095-2099 | ISSN:2251-6727

topical delivery systems for controlling inflammation in burn wounds.[14]

Effect of CTNG on Fibroblast Proliferation

Fibroblasts play a crucial role in wound healing during the proliferative phase through collagen synthesis, extracellular matrix production, and granulation tissue formation. The results of this study showed that the negative control group K (–) shows a significantly lower mean number of fibroblasts compared with the other burn-injured groups. This finding reflects an inhibition of the proliferative phase as a consequence of the strong inflammatory response following trauma.

The mean number of fibroblasts in the CTNG 2.5% (K2) and 5% (K3) groups is higher than that in the negative control K (–) and the K1 group treated with sulfadiazine. Notably, the K3 group shows the highest fibroblast count among all groups. These findings indicate that CTNG is not only anti-inflammatory but also supports tissue regeneration. This effect is associated with the ability of bioactive compounds in *Clitoria ternatea* to stimulate fibroblast migration and proliferation, as well as to improve the wound microenvironment through their antioxidant activity.[15]

Specifically, the nanogel formulation allows better penetration of active compounds into the dermal layer, thereby optimizing direct interactions with fibroblasts. This approach is consistent with reports indicating that topical nanotechnology-based systems can accelerate the transition from the inflammatory phase to the proliferative phase in burn wound healing. [16,6] the above findings are supported by histopathological observations shown in Figure 3, where the negative control group K (–) shows loosely arranged tissue with irregular collagen fibers, reflecting a failure of the proliferative phase. In contrast, the CTNG -treated groups, particularly the 5% concentration, shows a high density of fibroblasts and more organized collagen fibers, indicating a more advanced and mature wound-healing process.

Reduced inflammation makes it possible for fibroblast to optimally form granulation tissue and collagen, an initial phase of skin tissue remodeling [5]. Continuously high, IL-6 levels maintain pro-inflammatory (M1) macrophages, increase reactive oxygen species (ROS), and create a tissue environment that inhibits fibroblast migration and proliferation. Meanwhile, low IL-6 levels allow macrophages to polarize toward the reparative M2 phenotype, secreting growth factors such as TGF-β and PDGF to stimulate fibroblast proliferation and extracellular matrix synthesis [17]. These findings strengthen the potential role of CTNG as anti-inflammatory, stimulating burn wound healing. The healing is marked with low average level of IL-6, high number of fibroblast cells, and improvement of the dermal tissue appearance.

ACKNOWLEDGEMENT

The authors would like to thank Sultan Agung Islamic University for its support of this study.

CONFLICT OF INTEREST AND FUNDING SOURCES

The authors declare no conflict of interest, and this study was funded by the authors' personal funds.

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JCHR (2026) 16(2), 2095-2099 | ISSN:2251-6727

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