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Ruslan Aminov**EFFECT OF LEECH EXTRACT ON LYMPHOCYTES IN THERMAL BURNS****Руслан Амінов****ВПЛИВ ЕСТРАКТУ П'ЯВКИ НА ЛІМФОЦИТИ ПРИ ТЕРМІЧНИХ ОПІКАХ****ABSTRACT**

Purpose of the work. The study aimed to evaluate changes in the quantitative composition of lymphocytes and the blast transformation reaction of lymphocytes under spontaneous conditions and concanavalin A stimulation during the healing of a thermal burn, as well as to determine the immunomodulatory effect of the water-salt extract of the medicinal leech (*Hirudo verbana*).

Methodology. The experimental study was conducted using a thermal burn model. Animals were divided into intact, control (thermal burn without treatment), and experimental groups treated with the water-salt extract of *Hirudo verbana*. Blood samples were collected on days 3, 7, 14, and 30 after injury. The percentage of lymphocytes and the blast transformation reaction of lymphocytes were assessed under spontaneous conditions and after stimulation with concanavalin A using standard immunological methods. Results were expressed as $M \pm m$, and statistical analysis was performed using Student's t-test.

Scientific novelty. For the first time, the dynamics of lymphocyte proliferative activity during thermal burn healing under the influence of the water-salt extract of the medicinal leech were quantitatively characterized. It was demonstrated that the extract promotes faster restoration of ConA-induced blast transformation of lymphocytes and prevents prolonged post-burn immunosuppression.

Conclusions. Thermal burn injury caused transient lymphopenia, increased spontaneous blast transformation, and suppression of mitogen-induced lymphocyte proliferation. Application of the water-salt extract of *Hirudo verbana* reduced the severity of these changes and ensured normalization of both quantitative and functional lymphocyte parameters by day 14 of the experiment. The obtained results confirm the pronounced immunomodulatory potential of the medicinal leech extract during reparative processes.

Key words: lymphocytes, blast transformation, thermal burn, leech extract, immunomodulation

АНОТАЦІЯ

Мета роботи. Метою дослідження було оцінити зміни кількісного вмісту лімфоцитів та реакції бластної трансформації лімфоцитів за спонтанних умов і при стимуляції конканаваліном А в процесі загоєння термічного опіку, а також визначити імуномодулювальний вплив водно-сольового екстракту медичної п'явки (*Hirudo verbana*).

Методологія. Дослідження проведено на експериментальній моделі термічного опіку. Тварин розподілили на інтактну, контрольну (термічний опік без лікування) та дослідну групи, у якій застосовували водно-сольовий екстракт *Hirudo verbana*. Забір матеріалу здійснювали на 0, 3, 7, 14 та 30 добу після травми. Визначали відсотковий вміст лімфоцитів і реакцію бластної трансформації лімфоцитів у спонтанних умовах та після стимуляції конканаваліном А за стандартними імунологічними методиками. Статистичну обробку результатів проводили з використанням критерію Стьюдента.

Наукова новизна. Уперше кількісно охарактеризовано динаміку проліферативної активності лімфоцитів у процесі загоєння термічного опіку за умов застосування водно-сольового екстракту медичної п'явки. Показано, що екстракт сприяє ранньому відновленню КонА-індукованої бластної трансформації лімфоцитів та зменшує тривалість постопікової імунної дисфункції.

Висновки. Термічний опік супроводжується розвитком транзиторної лімфопенії, підвищенням спонтанної проліферації та пригніченням мітоген-індукованої активності лімфоцитів. Застосування водно-сольового екстракту *Hirudo verbana* забезпечує нормалізацію кількісних і функціональних показників лімфоцитарної ланки імунітету вже до 14 доби, що свідчить про його виражений імуномодулювальний ефект.

Ключові слова: лімфоцити, бластна трансформація, термічний опік, екстракт п'явки, імуномодуляція

Introduction

Thermal burn injury induces a complex systemic immune response that influences wound healing and susceptibility to complications such as infection, systemic inflammatory response syndrome (SIRS), and immunosuppression (Burgess et al., 2022). Burns trigger an initial pro-inflammatory phase driven by innate immune cells, followed by compensatory anti-inflammatory mechanisms that may lead to transient impairment of adaptive immunity, including suppression of T-lymphocyte function and reduction in lymphocyte counts (Dincer et al., 2025; Mulder et al., 2022). Quantitative and functional evaluation of lymphocytes, including blast transformation reactions under spontaneous conditions and after stimulation with mitogens such as concanavalin A (ConA), provides crucial insights into the cellular immune status after injury.

The blast transformation reaction of lymphocytes is an established method for assessing proliferative responses of T-cells. Changes in spontaneous and mitogen-induced blast transformation can reflect T-cell responsiveness and is used to detect immunosuppression in pathological conditions (Simon-Molas et al., 2021). In addition, clinical and experimental evidence suggests that severe burns create a prolonged inflammatory milieu, with elevated cytokines and immune cell recruitment, which may delay the resolution of inflammation and impair adaptive immune processes (Mulder et al., 2022).

Natural biologically active compounds with anti-inflammatory and immunomodulatory properties are increasingly studied for their potential to support immune regulation and tissue repair. Medicinal leech (*Hirudo verbana*) saliva and extracts contain diverse bioactive molecules, including anticoagulants, protease inhibitors, and anti-inflammatory peptides, which have been shown to modulate cytokine production and macrophage activation, suggesting a potential immunomodulatory role in injury contexts (Amani et al., 2021; Zakian et al., 2022; Ünal et al., 2023; Ayhan et al., 2024; Aminov et al., 2021,2022,2023,2025;). While these effects have been documented primarily in vitro, their influence on post-burn immune dysregulation

and lymphocyte function is not yet fully characterized.

Therefore, further investigation into both quantitative and functional changes in lymphocytes under the influence of leech-derived extracts during burn healing is essential to clarify their therapeutic potential and mechanisms of action.

The aim of this study was to evaluate changes in the percentage of lymphocytes and the blast transformation reaction of lymphocytes under spontaneous conditions and ConA stimulation during thermal burn healing, and to assess the immunomodulatory effects of a water-salt extract of *Hirudo verbana*.

Materials and methods

Preparation of aqueous-saline leech extract

For the preparation of the aqueous-saline extract, 50 specimens of medicinal leeches (*Hirudo verbana* Carena, 1820) were used. The leeches were bred at the Educational and Research Laboratory of Cellular and Organismal Biotechnology of Zaporizhzhia National University according to technical specifications TU U 05.0-02125243-002:2009 "Medicinal leech" and had a valid sanitary and epidemiological certificate (Ministry of Health of Ukraine No. 05.03.02-06/49982, 12.08.2009).

The final extract concentration was 0.022 mg/mL, and protein content was determined by colorimetric assay using pyrogallol red and molybdate on a Beckman Coulter AU480 biochemical analyzer (Aminov et al., 2025).

Experimental animals and housing conditions

The study included 80 outbred white laboratory rats weighing 260–300 g. Animals were housed individually in standard cages under controlled conditions (temperature 20–25 °C, relative humidity ≤ 55 %, natural light-dark cycle) and fed a balanced diet. Throughout the experiment, no signs of bacterial infection were detected.

Experimental design

Rats were randomly assigned to two groups (n = 40 each):

1. Control group – spontaneous wound healing without treatment;
2. Experimental group – daily topical applications of the aqueous-saline extract of *Hirudo verbana*.

All experimental manipulations complied with the International Guidelines for Biomedical Research Involving Animals, the national Joint Ethical Principles for Animal Experiments (Ukraine, 2001), and Directive 2010/63/EU of the European Parliament and of the Council (22 September 2010). The protocol was approved by the Bioethics Commission of the Zaporizhzhia National University (protocol no. 1, October 30, 2025).

Thermal burn modeling

Second-degree (II–III) thermal burns were induced on a shaved dorsal area under ketamine anesthesia (50 mg/kg, intraperitoneally). A copper plate (1.6 × 5 cm) heated to 100 °C ± 2 °C was applied for 10 seconds to create a standardized burn injury.

Treatment and sample collection

Treatment began 24 hours after burn induction and was applied once daily until complete healing. Blood samples were collected from the tail vein on days 0 (intact), 3, 7, 14, and 30 after injury.

Peripheral blood lymphocytes were analyzed in whole blood samples without prior cell isolation.

The percentage of lymphocytes was determined using standard hematological analyzers and confirmed by microscopic examination of Giemsa-stained blood smears.

The blast transformation reaction (BTR) was assessed in whole blood under spontaneous conditions and after stimulation with concanavalin A (ConA, 10 µg/mL). Blood samples were incubated at 37 °C for 24 hours, after which the percentage of blast-transformed lymphocytes was determined by cytomorphological analysis of stained blood smears.

Results were expressed as the percentage of lymphocytes and the percentage of blast-transformed lymphocytes.

Statistical analysis

Data are presented as mean ± SD. Comparisons were performed:

Between intact and control groups, and intact and experimental groups at each time point;

Between control and experimental groups at corresponding time points.

Normality was verified with the one-sample Kolmogorov–Smirnov test. Parametric comparisons were conducted using Student's t-test with Bonferroni correction. Analysis was performed using IBM SPSS Statistics 21.0 (IBM, USA). Differences were considered significant at $p < 0.05$.

Results and Discussion

The results of the present study demonstrated that thermal burn trauma significantly altered both the quantitative and functional parameters of peripheral blood lymphocytes. In the control group, lymphocyte percentage significantly decreased by day 3 after injury (61.8 ± 1.2 % vs intact 73.91 ± 1.0 %), indicating early lymphopenia – a hallmark of burn-induced immunosuppression noted in multiple studies of burn immunology (Burgess et al., 2022; Osuka et al., 2024). This early lymphocyte drop reflects the complex systemic immune response triggered by thermal damage, which involves not only inflammatory activation but also regulatory suppression of adaptive immunity (Osuka et al., 2024).

In control animals the spontaneous blast transformation (SP) of lymphocytes increased transiently on day 3, whereas ConA-induced blast transformation showed a marked decrease (11.6 ± 0.8 % vs intact 19.6 ± 1.1 %). Concanavalin A (ConA) is a widely used plant mitogen that induces T-cell proliferation and is considered a robust indicator of lymphocyte functional capacity in immune assays (Simon-Molas et al., 2021). The suppression of ConA-induced blast transformation in the control group is consistent with observed functional impairment of T cell responsiveness early after burn, as part of the mixed compensatory anti-inflammatory response syndrome (CARS) and persistent immunosuppression post-burn (Osuka et al., 2024; Korkmaz, 2023).

Animals treated with the water-salt extract of *Hirudo verbana* showed higher lymphocyte percentages at day 3 (67.9 ± 1.1 %) and improved ConA-stimulated blast transformation (15.8 ± 0.9 %) compared with controls. This suggests that the extract mitigated early post-burn immune suppression, facilitating both preservation of lymphocyte counts and maintenance of proliferative responsiveness. The immunomodulatory potential of leech-derived bioactive compounds has been documented in recent studies showing anti-inflammatory and immune-regulating effects in vitro, including down-regulation of pro-inflammatory cytokine production in activated immune cells (Ayhan et al., 2024).

At later time points (days 7–30), experimental animals continued to demonstrate accelerated restoration of immune parameters compared with controls. By day 30, the experimental group had lymphocyte percentages ($75.2 \pm 0.8\%$) and ConA-induced blast transformation ($21.3 \pm 1\%$) exceeding intact values, whereas controls did not fully reach baseline. These trends suggest that the leech extract promotes not only earlier recovery but potentially enhanced adaptive responsiveness beyond normal levels. Such enhanced recovery may involve modulation of inflammatory signaling, attenuation of excessive pro-inflammatory cascades, and support of T-cell regenerative

processes, aligning with broader evidence that persistent systemic inflammation and immune dysregulation are key barriers to immune restoration post-burn (Mulder et al., 2021; Korkmaz, 2023).

Overall, these data support the view that thermal burns induce complex immune changes – initially driven by systemic inflammation and subsequent regulatory suppression – and that immunomodulatory interventions like *Hirudo verbana* extracts may support the restoration of cellular immunity by preserving lymphocyte numbers and functional responsiveness to mitogenic stimulation.

Table 1

Total percentage of lymphocytes and blast transformation reaction of rat blood lymphocytes

Animal group	Lymphocytes, %	Type of lymphocyte stimulation	Blast-transformed lymphocytes, %
Intact	73.91 ± 1.0	Spontaneous	9.3 ± 0.5
		ConA	$19.6 \pm 1.1^*$
Day 3 – Control	$61.8 \pm 1.2^*$	Spontaneous	$13.8 \pm 0.7^*$
		ConA	$11.6 \pm 0.8^*$
Day 3 – Experimental	$67.9 \pm 1.1^*$	Spontaneous	$11.2 \pm 0.6^*$
		ConA	$15.8 \pm 0.9^*$
Day 7 – Control	$65.4 \pm 1.1^*$	Spontaneous	$12.1 \pm 0.6^*$
		ConA	$14.2 \pm 0.9^*$
Day 7 – Experimental	71.8 ± 1.0	Spontaneous	10.1 ± 0.5
		ConA	18.6 ± 1.0
Day 14 – Control	69.1 ± 1.0	Spontaneous	10.6 ± 0.5
		ConA	17.1 ± 1.0
Day 14 – Experimental	74.6 ± 0.9	Spontaneous	9.5 ± 0.4
		ConA	20.9 ± 1.1
Day 30 – Control	71.0 ± 0.9	Spontaneous	9.8 ± 0.4
		ConA	18.0 ± 0.9
Day 30 – Experimental	75.2 ± 0.8	Spontaneous	9.2 ± 0.3
		ConA	21.3 ± 1.0

Note: ConA – blast transformation reaction of lymphocytes with lectin; % – relative value; * – $p < 0.05$, values significantly different from the spontaneous reaction

Conclusions

Thermal burn injury causes suppression of cellular immunity, manifested as decreased lymphocyte counts and impaired T-cell proliferative responses. The aqueous-saline extract of *Hirudo verbana* positively modulates the immune response, supporting both

lymphocyte numbers and functional activity. Treatment with the extract accelerates recovery of cellular immunity, which may contribute to improved wound healing and reduced post-burn complications. Leech-derived extracts have potential as immunomodulatory agents in post-burn therapy, warranting further investigation.

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Заява про доступність даних / Data Availability Statement

Набір даних доступний за запитом до автора / Dataset available on request from the author.

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