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# THE EFFECT OF THE MEDICINAL COMPOSITION "EFLORNITHINE-ARMENICUM" ON THE PROGRESSION OF THE INFLAMMATORY PROCESS IN AN EXPERIMENTALLY INDUCED AEROBIC WOUND

**GHAZARYAN H.V.**

Arpimed Pharmaceutical Company LLC, Abovyan, Armenia

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***ABSTRACT***

*Wound infection remains one of the most serious current challenges in modern medicine. Significant challenges in the symptomatic and pathogenetic therapy of wound infections arise due to the known symbiosis between pathogenic and opportunistic bacteria and certain pathogenic and opportunistic fungi. The mixed bacterial-fungal microflora persisting during a wound infection is often described as a biofilm. Notably, the addition of a fungal infection significantly worsens the wound healing process: on one hand, fungi that persist in the host's wound are inherently toxic; on the other, their association with bacteria often enhances the pathogenic potential of the bacteria.*

*The therapeutic efficacy of the medicinal composition Eflornithine-Armenicum was studied using an experimentally induced aerobic wound model. This medicinal composition was developed at the Research Center of the Yerevan State Medical University in collaboration with Arpimed LLC.*

*A wide range of morphological, morphometric, cytological, bacteriostatic, and immunomorphological studies were conducted. It was found that three applications of the composition to the wound surface on the skin of experimental rats led to an early activation of reparative and proliferative processes, ultimately resulting in complete restoration of the integrity of the damaged wound tissues through substitution.*

*The therapeutic effectiveness of the tested medicinal composition is, on one hand, due to the pronounced antibacterial activity of Eflornithine, which facilitated the early self-cleansing of the wound from opportunistic and pathogenic microorganisms persisting in situ. On the other hand, the effectiveness is attributed to the strong anti-inflammatory activity of Armenicum paste, thanks to the presence of ionized iodine in its composition.*

*Based on our studies, we believe there are broad prospects for further preclinical and clinical research on Eflornithine-Armenicum as an effective therapeutic agent for the pathogenetic treatment of wound inflammation.*

*Based on our comprehensive studies, we conclude that the medicinal composition Eflornithine-Armenicum, which we developed, should be considered an effective therapeutic agent in the treatment of aerobic wounds. This is particularly important, as both components of the composition have long been approved by prestigious pharmaceutical regulatory bodies as medicinal products with confirmed effectiveness and safety.*

***KEYWORDS:*** *aerobic wound, wound infections, medicinal composition "Eflornithine-Armenicum, treatment.*

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***ADDRESS FOR CORRESPONDENCE:***

Hovhannes V. Ghazaryan
Arpimed Pharmaceutical Company LLC
2nd Micro-District, 19 b., Abovyan 2204, Kotayk Marz, Armenia
Tel.: (374 41) 020510
E-mail: hovhannes.ghazaryan@arpimed.com

### *INTRODUCTION*

Wound infections remain a significant and serious problem in modern medicine. The situation is further complicated by the changing nature of pathological processes and the resistance of opportunistic and pathogenic microorganisms in wounds to many antibacterial drugs, especially antibiotics [*Pfaller M, 2012, Kapoor G et al., 2017; Reygaert W, 2018; Berman J, Krysan D, 2020*].

Significant challenges in symptomatic and pathogenetic therapy during wound infection arise from the known symbiosis of pathogenic and opportunistic bacteria, as well as certain fungi. This mixed bacterial-fungal microflora in wound infections is often described as a biofilm [*James G et al., 2008, Dowd S et al., 2011; Clinton A, Carter T, 2015; Percival S et al., 2015, Michael AJ, 2018, Rocha R, Wilson R, 2018*]. It is particularly noteworthy that the addition of a fungal infection significantly worsens the progression of the wound healing process. On one hand, the fungi persisting in the host wound are inherently toxic; on the other, when in association with bacteria, they often enhance the pathogenic potential of the bacterial presence. Unfortunately, current antifungal therapies are not always effective [*Becker W, 1991, Pruskowski K et al., 2021*].

The current situation regarding daily diet choices is concerning, as various supplements, including hormonal ones, are commonly used to increase the meat mass of poultry, fish, cattle and small ruminants [*Fritsche S, Steinhart H, 1999, Saha S, Pathak N, 2021*].

As a result of the factors mentioned above, the search for effective agents, and particularly the development of medicinal compositions with a broad, multipotent spectrum of action, represents a promising and relevant scientific and practical approach in modern medicine.

At the research center of Yerevan State Medical University named after M. Herats and Arpimed, LLC (Abovyan, Armenia), we have developed and successfully completed preclinical testing of the medicinal composition “Eflornithine (DFMO)-Armenicum” [*Ghazaryan H, Hovhannisyan A, 2022*].

The selection of agents for the medicinal composition was determined by the following consideration. It is well established that DFMO exhibits a strong inhibitory effect, suppressing the synthesis of aliphatic polyamines at the earliest stages of their enzymatic transformation, specifically, the conversion of ornithine to putrescine [*Meyskens F, Gerner E, 1999; Gerner E, Meyskens F, 2009*].

It is also worth noting that in recent years, highly informative data has emerged indicating that the vital activity and persistence of many resident pathogenic and opportunistic bacteria, viruses, and fungi within the host body are largely maintained by mechanisms that are specifically polyamine-dependent [*Wallace H, Fraser A, 2004; Shah P, Swiatlo E, 2008; Wallace H, 2009; Valdés-Santiago L et al., 2012; Valdés-Santiago L, Ruiz-Herrera J, 2014; Bae D et al., 2018; Berman J, Krysan D, 2020*]. In this context, there have been rare attempts to use DFMO to inhibit the synthesis of aliphatic polyamines not only in somatic cells but also to suppress their synthesis within microbial cells [*Wallace H, Fraser A, 2004; Wallace H, 2009; Bae D et al., 2018; Berman J, Krysan D, 2020*].

The second component of the medicinal composition, *Armenicum* paste, has a notably pronounced anti-inflammatory and partially bacteriostatic spectrum of action [*Zilfyan A et al., 2016*].

### *MATERIAL AND METHODS*

The investigation involved 180 male Wistar rats, in which an aerobic wound model was induced according to the method proposed by Hovhannisyan S.S. et al., for which a patent has been granted [*Hovhannisyan S et al., 1987*].

The animals in both the experimental and control groups were divided into three subgroups, which were removed from the experiment on the third, fifth, and ninth days of the investigation. The control group received only *Armenicum* paste, applied to the wound surface three times at 4-hour intervals, at a dose of 5.1 *mg/kg*. In addition to *Armenicum* paste, the experimental group also received the medicinal composition Eflornithine (DFMO)-*Armenicum*, applied to the wound surface three times at the same intervals. A single dose of *Armenicum* paste was 5.1 *mg/kg*, while a single dose of Eflornithine was 460 *mg/kg*.

The study employed conventional morphological methods, including staining with azure-II eosin and hematoxylin-eosin. Bacteriological methods were also used, incorporating staining with azure-II eosin and fluorochromizing with acridine orange.

An immunomorphological method was utilized to detect fibronectin in tissues, specifically the indirect Coons method with rabbit anti-fibronectin serum (Sigma, USA) and FITC-labeled anti-rabbit IgG serum (Sigma, USA). The preparations were examined under a trinocular light microscope (Micros, Austria) and a trinocular fluorescence microscope (Boeco, Germany).

Statistical analysis was performed using the SPSS program, 13 ANOVA version, using Student's t-criteria.

### *Results and discussion*

The study results indicated that local treatment of skin wounds in the experimental rats led to a marked activation of local reparative-proliferative processes. In contrast to the control group, which received only *Armenicum* paste, the experimental group showed activation of reparative-proliferative processes as early as the third day of the investigation. In the control group, where only *Armenicum* paste was applied to the wound surface, processes aimed at restoring defect integrity were observed only on the fifth and ninth days of the regional inflammatory process.

By the third day of the experiment, reparative processes were evident through the focal development of granulation tissue, which by the fifth day had become more widespread and showed increased differentiation (Fig. 1 a, b, c, d).

A clear trend toward differentiation of granu-

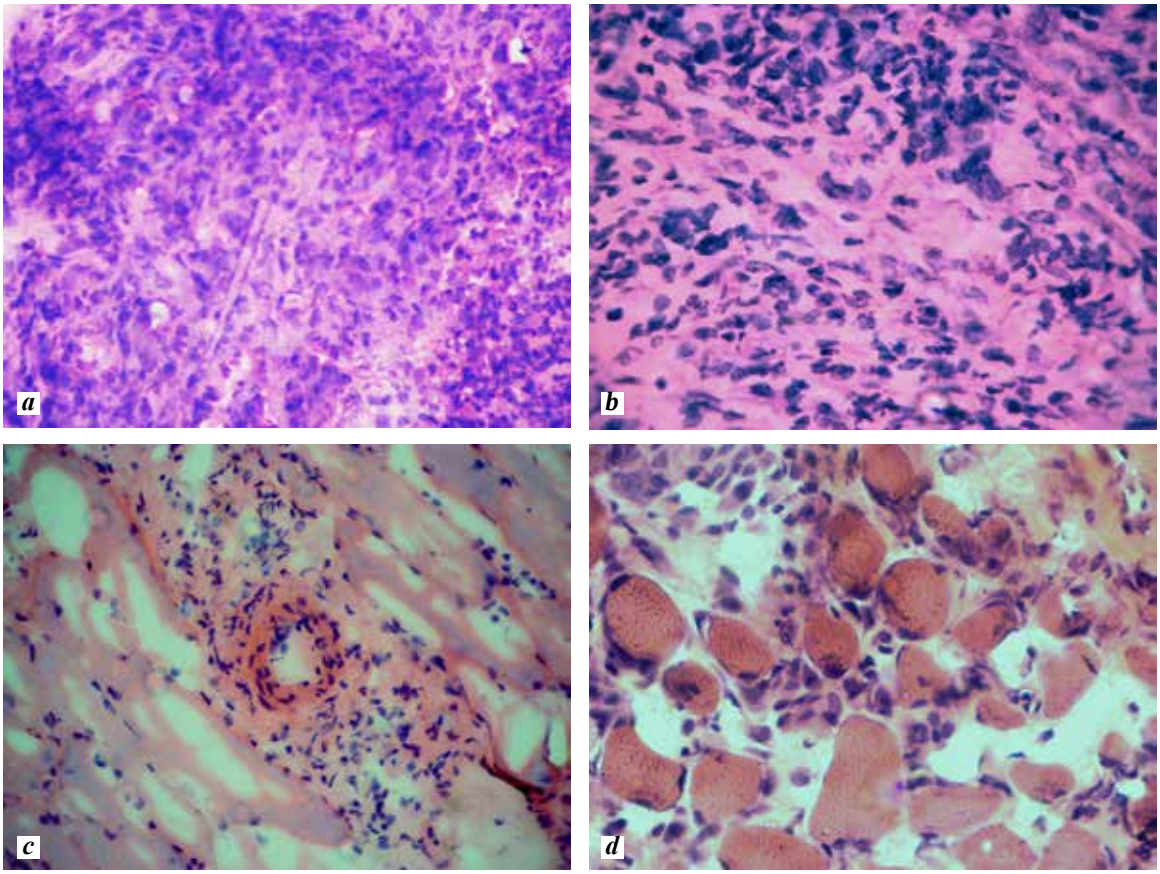


***Figure 1.*** *Structural changes in the soft tissues of the wound in animals from the experimental group on the 5th day of investigation.*

*(a). Further differentiation of granulation tissue transitioning to loose connective tissue in the superficial areas of the wound. Hematoxylin-eosin staining. Oc. 15, Ob. 60.*

*(b). Against a background of moderate edema, signs of granulation tissue growth with a tendency toward an organized structure of newly formed collagen fibers are observed. Stained with azure II-eosin. Oc. 15, Ob. 60.*

*(c). Productive subacute vasculitis with perivascular myocytolysis in the underlying muscle tissue. Stained with hematoxylin - eosin. Oc. 10, Ob. 10.*

*(d). Edema and cellular infiltration of the intermuscular tissue, with dystrophic changes observed in a distinct group of myocytes. Stained with hematoxylin and eosin. Oc 10, Ob. 60.*

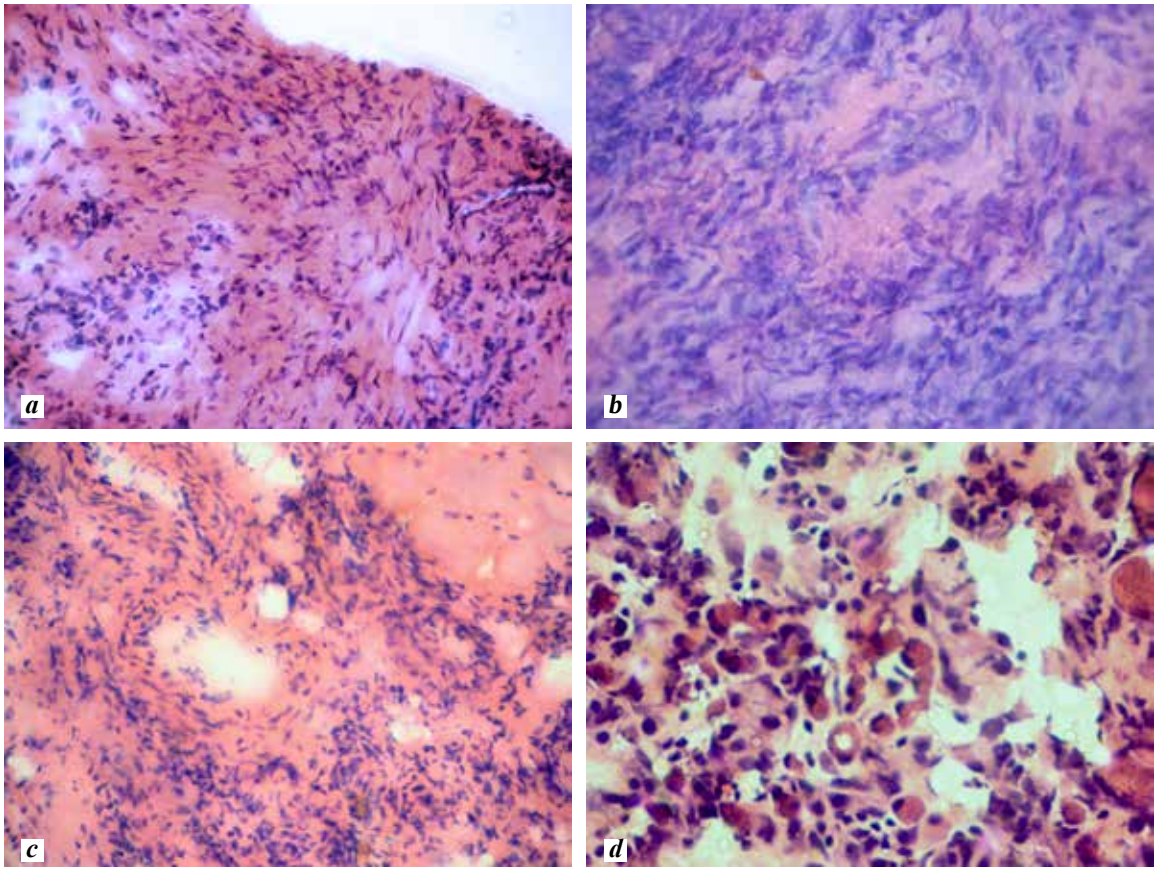


***Figure 2.*** *Structural changes in the soft tissues of the wound in control group animals. Stained with hematoxylin - eosin. 5th day of investigation.*

*(a). Dystrophic changes in inflammatory and connective tissue cells in the superficial areas of the wound, with early focal signs of granulation tissue revitalization in these regions. Oc. 15, Ob. 20.*

*(b). Poorly differentiated strands of granulation tissue with randomly oriented collagen fibers. Oc. 15, Ob. 20.*

(c). Moderate perivascular infiltration of inflammatory cells, with focal activation of fibroblastic cells. Oc. 15, Ob. 10.

(d). Single muscle cells are visible in cross-section. The intermuscular spaces are compressed and infiltrated with inflammatory cells. Oc. 15, Ob. 60.

lation tissue was observed. In the control group animals, granulation tissue remained detectable even at the later stages of the investigation. Additionally, catabolic processes were observed more frequently in the control group, marked by areas of necrobiosis and death of connective elements, including fibroblastic and angiomatous cells, as well as myocytes (Fig. 2 a, b, c, d).

At relatively late stages of the regional inflammatory process (the 9th day of investigation), the wound induced in rats healed through substitution, meaning the integrity of the soft tissue covering the wound defect was restored by secondary intention and connective tissue growth (Fig. 3 a, b).

What are the possible mechanisms underlying the beneficial effect of the medicinal composition "Eflornithine-Armenicum" on the recovery process in an induced aerobic wound?

Primarily, the positive effect of local application of this medicinal composition can be attributed to the direct action of its components on the bacterial landscape of the wound. Notably, the composition exhibited a pronounced bactericidal effect, simultaneously targeting both pathogenic and opportunistic microflora persisting in the wound. This observation is supported by our bacterioscopic analysis of the wound exudate microflora. As mentioned earlier, many opportunistic and pathogenic microorganisms, including bacteria residing in the wound exudate and soft tissues, particularly in areas of tissue destruction, require aliphatic polyamines to support their reproduction and vital functions.

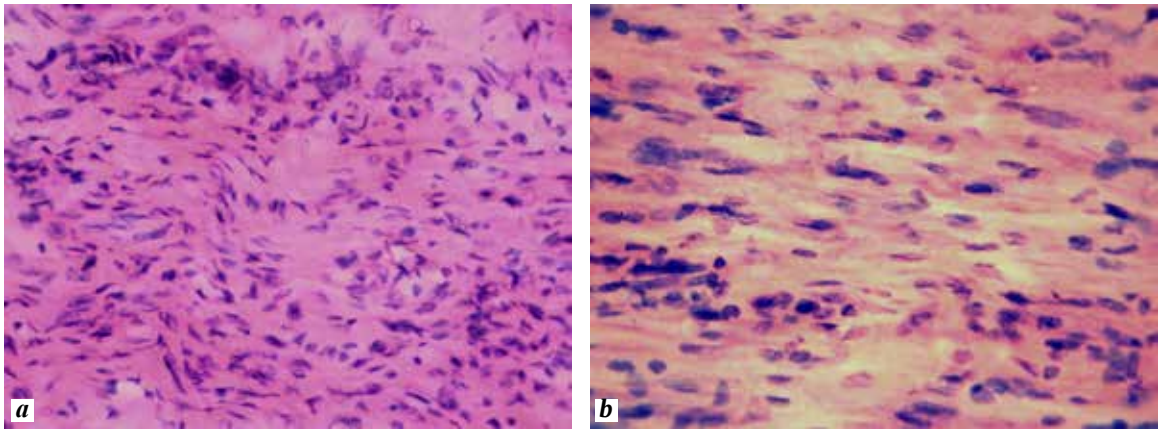


***Figure 3.*** *Structural changes in the soft tissues of the wound in experimental group animals. 9th day of investigation.*
*(a). Differentiated connective tissue is present in the superficial parts of the wound. Collagen fibers acquire a linear, ordered orientation, with mature fibrocytes beginning to predominate among the fibroblastic cells. Stained with hematoxylin - eosin. Oc 15, Ob. 20.*
*(b). Further differentiation of granulation tissue in the deep parts of the wound. Stained with hematoxylin and eosin. Oc. 15, Ob. 10.*

It is particularly noteworthy that the pronounced bactericidal effect observed was primarily due to DFMO in the medicinal composition, which facilitated rapid cleansing of the wound from persisting microorganisms. This effect was confirmed by our cytological and bacterioscopic studies, using azure-II eosin and acridine orange staining (Fig. 4 a, b).

We also observed that, with the cleansing of the wound from microorganisms, the structural and functional characteristics of wound exudate cells, macrophages, leukocytes, and lymphocytes, became significantly normalized. Concurrently, complete phagocytosis was activated in structurally intact macrophages within the wound exudate. This facilitated early activation of reparative and proliferative processes in the soft tissue surrounding the wound, ultimately leading to full healing by substitution, wherein the entire length of the damaged tissues was replaced by loose connective tissue.

Additionally, our immunomorphological studies revealed that the presence of eflornithine in the medicinal composition promoted pronounced syn-

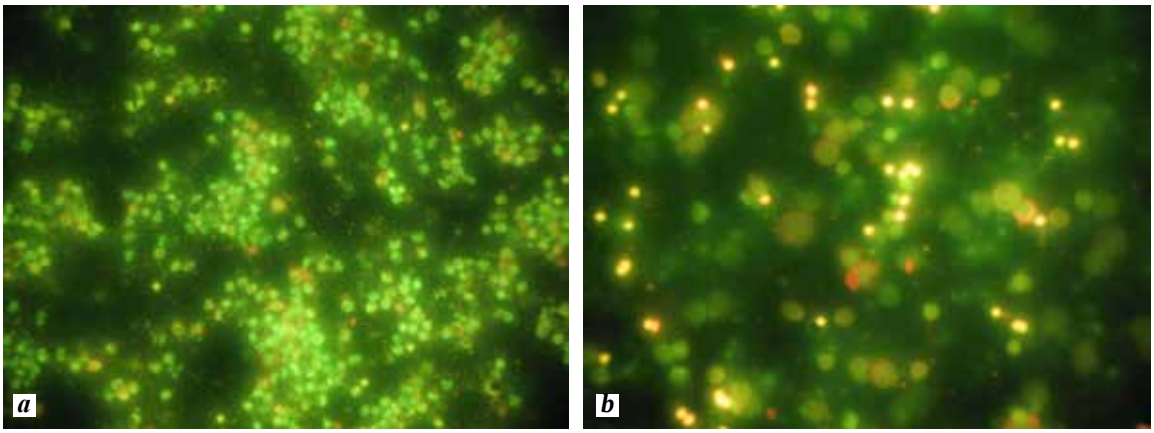


***Figure 4.*** *Structural changes in immunocompetent cells of wound exudate in experimental group animals. Stained with acridine orange. Oc. 15, Ob. 20*
*(a). Single macrophages and a moderate number of lympho-leukocyte cells are present, with changes in their tinctorial properties: green fluorescence shifts to orange-red. Extracellularly oriented single green and orange-red granules are also observed. 3rd day of investigation.*
*(b). Structurally preserved immunocompetent cells with green fluorescence dominate in the exudate. 5th day of investigation.*

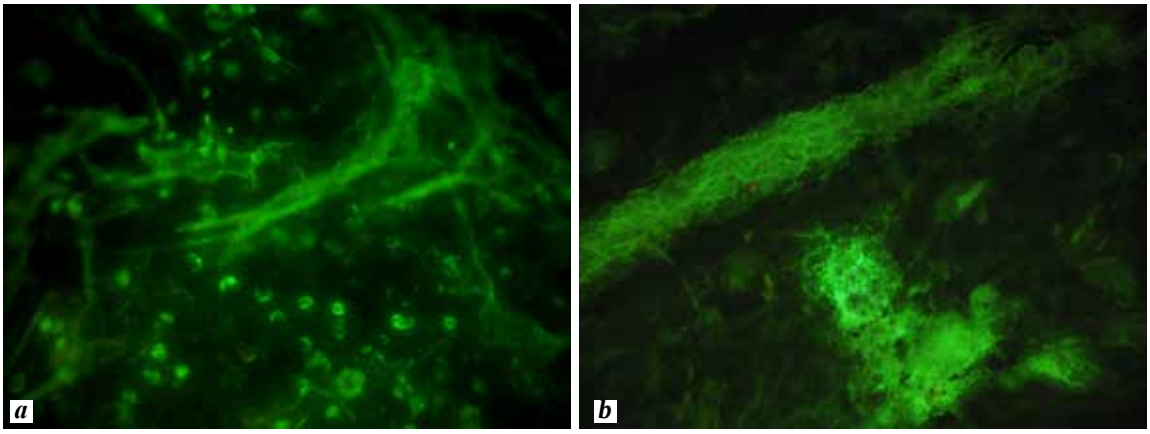


***Figure 5.*** *Presence of fibronectin in the soft tissues of the wound in experimental group animals. Luminescent microscopy, 3rd day of the experiment.*
*(a). Specific luminescence in the cytoplasm of fibroblast cells, indicating the presence of fibronectin. Oc. 15, Ob. 40.*
*(b). Fibronectin deposits in the superficial and deep layers of the wound's soft tissues. Oc. 10, Ob. 10.*

thesis of fibronectin by fibroblasts in the wound's soft tissues, even at the early stages of the inflammatory process (Fig. 5 a, b).

Fibronectin is a well-known potent activator of fibroblast cell proliferation, which, in turn, initiates the production of collagen protein structures during the wound inflammatory process [*Grinnell F et al., 1981; Lenselink E, 2015; Gimeno-LLuch I et al., 2022*]

### CONCLUSION

Based on our comprehensive studies, we conclude that the medicinal composition "*Eflornithine-Armenicum"* should be considered an effective therapeutic agent for treating aerobic wounds. This is particularly significant, as both components have been approved by prestigious pharmaceutical regulatory bodies, affirming their effectiveness and safety as medicinal products.

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**Rector of YSMU**

Armen A. Muradyan



**Address for correspondence:**

Yerevan State Medical University
2 Koryun Street, Yerevan 0025,
Republic of Armenia

**Phones:**

(+37410) 582532 YSMU
(+37493 588697 Editor-in-Chief
**Fax:** (+37410) 582532
**E-mail:**namj.ysmu@gmail.com, ysmiu@mail.ru
**URL:**http//www.ysmu.am



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Director: Armen Armenaakyan
Andraniks St., 96/8 Bulding
Yerevan, 0064, Armenia
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