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Wound-Healing effect of substances based on lanolin and *Ganoderma applanatum* and *Fomitopsis pinicola* (xylotrophic basidiomycetes) extracts

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ABSTRACT

Aim. To evaluate the wound-healing effect of substances based on lanolin and *Ganoderma applanatum* and *Fomitopsis pinicola* extracts.

Materials and methods. The experiment used 20 white laboratory rats. The study included 3rd-degree burn modeling with wound exposure to an ointment containing *Ganoderma applanatum* and *Fomitopsis pinicola* extracts. A morphological investigation of skin was carried out with an assessment of the following parameters: epidermis thickness, fibroblast count, number of blood vessels, vessel area, and number of vessels with erythrocyte stasis, leukocyte stasis, and leukocyte diapedesis.

Results. *Ganoderma applanatum* and *Fomitopsis pinicola* extracts intensified regenerative processes. By day 14 of the experiment, epithelialization of the defect and scar formation took place. Application of the extract-containing ointments reduced exudation, microcirculatory disorders, and inflammatory infiltration.

Conclusion. The results suggested the beneficial effect of the *Ganoderma applanatum* and *Fomitopsis pinicola* extracts on the wound-healing process and demonstrated their potential for skin regeneration.

Keywords: skin regeneration, burn wounds, wound-healing agents, xylotrophic basidiomycetes, *Ganoderma* extract, *Fomitopsis* extract

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Оценка ранозаживляющего действия субстанций на основе ланолина и экстрактов ксилотрофных базидиомицетов *Ganoderma applanatum* и *Fomitopsis pinicola*

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РЕЗЮМЕ

Цель: оценить ранозаживляющее действие субстанций на основе ланолина и экстрактов ксилотрофных базидиомицетов *Ganoderma applanatum* и *Fomitopsis pinicola.*

Материалы и методы. Эксперимент проводили на 20 белых лабораторных крысах. Животным моделировали термический ожог III степени с последующим воздействием на раны мазью, содержащей экстракты трутовых грибов. Проводили морфологическое описание препаратов кожи с оценкой следующих показателей: толщина эпидермиса, количество фибробластов, количество кровеносных сосудов, площадь сосудов, количество сосудов с явлениями эритростаза, лейкостаза и лейкодиапедеза.

Результаты. При воздействии экстрактов трутовых грибов отмечается интенсификация регенераторных процессов. К 14-м сут эксперимента имела место полная эпителизация, а на месте дефекта формировался рубец с развитым волокнистым компонентом. Использование мазей способствует уменьшению экссудативных явлений, микроциркуляторных расстройств и воспалительной инфильтрации.

Заключение. Показано, что использование экстрактов *Ganoderma applanatum* и *Fomitopsis pinicola* при лечении ожоговой раны благоприятно сказывается на течении раневого процесса. Полученные результаты позволяют осуществлять дальнейшее изучение экстрактов трутовых грибов в качестве ранозаживляющих агентов.

Ключевые слова: регенерация кожи, ожоговые раны, ранозаживляющие средства, ксилотрофные базидиомицеты, экстракт *Ganoderma*, экстракт *Fomitopsis*

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы декларируют отсутствие внешнего финансирования для проведения исследования и публикации статьи.

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INTRODUCTION

In recent years, researchers have increasingly focused on discovering natural medicinal compounds. In particular, studies demonstrating the wound-healing effects of preparations derived from polypores have emerged [1]. Xylotrophic basidiomycetes (wood-decaying polypores) represent a vast but largely untapped source of novel pharmaceutical products. The most frequently cited polypore in traditional medicine, *Ganoderma lucidum*, has been used as a therapeutic agent in China for over two millennia [2]. Modern studies confirm that preparations derived from *Ganoderma lucidum* exhibit hypoglycemic, anti-inflammatory, antitumor, and immunomodulatory properties [3, 4]. For another, less-studied species of the *Ganoderma* genus – *G. applanatum,* the presence of similar effects has been confirmed by more recent research [5, 6].

The long-standing use of *Ganoderma* fungi and current scientific interest in them stem from their rich content of metabolites: polysaccharides, phenolic compounds, alkaloids, sterols, flavonoids, amino acids, and others [7, 8]. The activity spectrum of these compounds is broad [9, 10] but occasionally contradictory. For instance, certain terpenes of *G. applanatum* exhibit anti-angiogenic effects [11], while lanostane triterpenoids demonstrate opposing pro-angiogenic effects [12].

In addition to polypores of the *Ganoderma* genus, fungi from the *Fomitopsis* genus have been widely used in traditional medicine, with *Fomitopsis pinicola* being one of its species [13]. However, *Fomitopsis* remains significantly understudied compared to the more extensively researched *Ganoderma*. Recent studies have identified new potential anti-inflammatory agents in *F. pinicola* fruiting bodies [14, 15].

In summary, both *G. applanatum* and *F. pinicola* are strong candidates for evaluating their effects on regeneration processes. The feasibility and safety of using xylotrophic macromycetes for developing wound-healing agents are further supported by the growing demand for raw materials from these fungi in cosmetology [16]. Thus, this study is aimed at assessing the wound-healing effects of substances based on lanolin and xylotrophic basidiomycetes *Ganoderma applanatum* and *Fomitopsis pinicola* extracts.

MATERIALS AND METHODS

The study was conducted on 20 outbred white laboratory rats (males, aged 6 months). All experiments were approved by the Bioethics Committee at the Institute of Natural Sciences and Mathematics, Ural Federal University. A third-degree burn was modeled by applying a medical steel weight (2 cm diameter, 20 g) heated to 100 °C to the interscapular skin area for 30 seconds. Prior to this, the animals received an intramuscular injection of Analgin (0.2 ml). The animals were randomly divided into 4 groups (n = 5):

CONTR, Control – rats with untreated wounds;

LAN, Lanolin – rats with wounds treated with lanolin;

LAN+extGND – rats with wounds treated with an ointment based on lanolin and a condensed alcoholic and aqueous *Ganoderma applanatum* extract;

LAN+extFP – rats with wounds treated with an ointment based on lanolin and a condensed alcoholic and aqueous *Fomitopsis pinicola* extract.

Ointments were applied to the wound area once daily (0.2 g) for 14 days. The animals were removed from the experiment on day 14 via diethyl ether overdose. Wounds were photographed with a Nikon D3000 camera on days 1, 3, 7, and 14. Wound areas were evaluated using the ImageJ software.

Fruiting bodies of *G. applanatum* and *F. pinicola* were collected in summer in a mixed forest in the vicinity of the Ural Federal University biological station (Russia, Sverdlovsk Region, the village of Klyuchi, 30 km south of Yekaterinburg). The specimens were dried in a shaded ventilated room for up to one week. Then the material was fixed by heating at 105 °C for 15 minutes to eliminate insect eggs and larvae and dried to constant mass at 50 °C. Extracts were prepared via sequential extraction with 95%, 70%, and 40% ethanol and distilled water (biomass : solvent ratio of 1:10; extraction time – 90 minutes at 50 °C). Combined extracts were evaporated on a rotary evaporator to aqueous residue, then adjusted with hydroalcoholic mixture. To prepare the 5% ointment, 7.5 ml of the extract (equivalent to 750 mg fungal biomass) was added at vigorous stirring to 7.5 g lanolin melted at 50 °C in a water bath until homogeneous consistency was achieved.

The chemical composition of the extracts was assessed by diluting stock suspension 20-fold with distilled water. Spectrophotometric methods were used to determine the content of phenolic compounds (via Folin – Ciocalteu's phenol reagent and 7.5 % sodium carbonate aqueous solution, with gallic acid as the standard), flavonoids (as complexes with aluminum chloride, with rutin as the standard), and free amino acids (with the ninhydrin reagent and glycine as the standard). The methods were adapted for the microplate immune-assay [17].

Extracted mass was determined gravimetrically by evaporating aliquots of stock suspension and recalculating the obtained value to ointment content. The qualitative analysis followed standard pharmacognostic methods. Saponins were determined by the foam formation in the aqueous solution and precipitation with lead acetate. Tannins were detected by the ferric (III) chloride test and precipitation test with potassium dichromate or lead acetate. Anthraquinones were determined in the color reaction with magnesium acetate. Dragendorff's, Marme's, Marquis, and Bouchardat's reagents were used for detecting alkaloids. Individual amino acids were identified by thin-layer chromatography (TLC) in n-butanol:acetic acid:water (4:1:1) [18]. All tests were performed in 4 analytical replicates.

For the histological analysis, standard procedures were employed. Tissue specimens (3 μm) were stained with hematoxylin and eosin and Van Gieson's picrofuchsin. The ToupView software was applied for the histological analysis. The epidermal thickness, fibroblast count, and vessel area and number (including some that exhibit stasis or diapedesis) were analyzed according to 10 randomly selected fields with conversion to 1 mm^2.

The statistical analysis was performed using Microsoft Excel, Mathematica 12.0, and Statistica 12.0 packages. Data sets were compared by the Kruskal – Wallis test with the Dunn's post-hoc analysis. The results were presented as the median and the interquartile range *Me* [Q_1; Q_3]. The values of $p < 0.05$ were considered statistically significant.

RESULTS

The table below summarizes the chemical composition of the tested extracts.

Table

Chemical Composition of the *Ganoderma Applanatum* and *Fomitopsis Pinicola* Extracts, *Me* [Q_1; Q_3]

Bioactive substances in 1 g ointments containing *Ganoderma applanatum* and *Fomitopsis pinicola* extracts									
Fungi species	Extractives, mg / ml	Phenolics, mg / ml	Flavonoids, mg / ml	Free amino acids		Qualitative composition			
				mg / ml	Quantity	Alkaloids	Saponins	Anthraquinones	Tannins
G. applanatum	4.5 [4.5 ; 4.9]	0.403 [0.398 ; 0.420]	0.008 [0.007 ; 0.009]	0.018 [0.015; 0.023]	6	-	-	-	-
F. pinicola	5.5 [5.5 ; 6.3]	0.42 [0.373 ; 0.432]	0.016 [0.015 ; 0.016]*	0.014 [0.013 ; 0.017]	6	+	+	–	-

* significant differences compared to the *G. applanatum* group ($p < 0.05$).

The comparative analysis revealed higher extractability for *F. pinicola*. Both extracts contained negligible free amino acids and carbohydrates (below detection limit via TLC or anthrone colorimetry). Phenolics accounted for no more than 10 % of total extracted substances, with similar levels in both extracts. Flavonoids were detected at higher concentrations than free amino acids. *F. pinicola* exhibited twice the flavonoid content of *G. applanatum*. Notably, flavonoids comprised a larger proportion of phenolics in *F. pinicola*. Alkaloids and saponins were also present in *F. pinicola* extracts, indicating their greater metabolic diversity.

Wound-healing dynamics (Fig. 1) showed accelerated healing in fungal extract-treated groups, with earlier crust formation and sloughing as well as wound closure. Thus, by the end of the experiment, a decrease in wound area in the LAN+extGND group significantly exceeded that of not only the control group, but also of the LAN group.

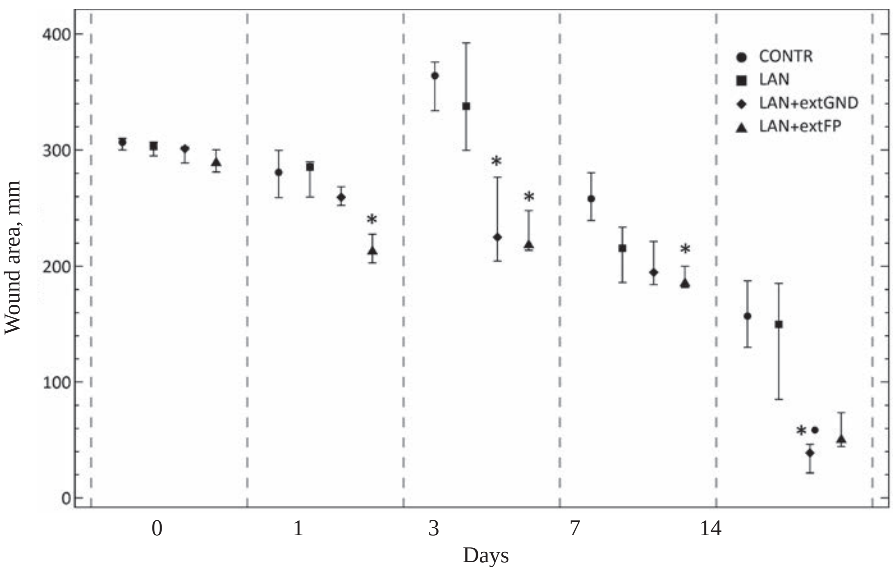

Fig. 1. Wound area reduction:
* significant differences compared to the CONTR group, $p < 0.05$;
● significant differences compared to the LAN group, $p < 0.05$

The histological analysis on day 14 revealed persistent leukocyte necrotic layers with fibrin clusters and damaged tissue in the CONTR and LAN groups. Epithelialization was limited to wound margins. Dermal infiltration and dilated vessels with stasis and diapedesis were observed (Fig. 2). Van Gieson's staining showed thin and disorganized pale crimson collagen bundles (Fig. 3).

In the LAN+extGND and LAN+extFP groups, the defect was closed by neo-epidermis with marked acanthosis, so that epidermis thickness increased compared to the control group (Fig. 4, *a*). Subjacent granulation tissue contained a large number of fibroblasts and neovessels. In the LAN+extGND group, inflammation was absent in most regenerated skin areas (Fig. 2). Massive twisted bundles of collagen fibers were detected deep in the regenerated skin, indicating intensive fibrous component development (Fig. 3). In the LAN+extFP group, moderate perivascular leukocyte infiltration persisted (Fig. 2) with less pronounced fiber formation (Fig. 3).

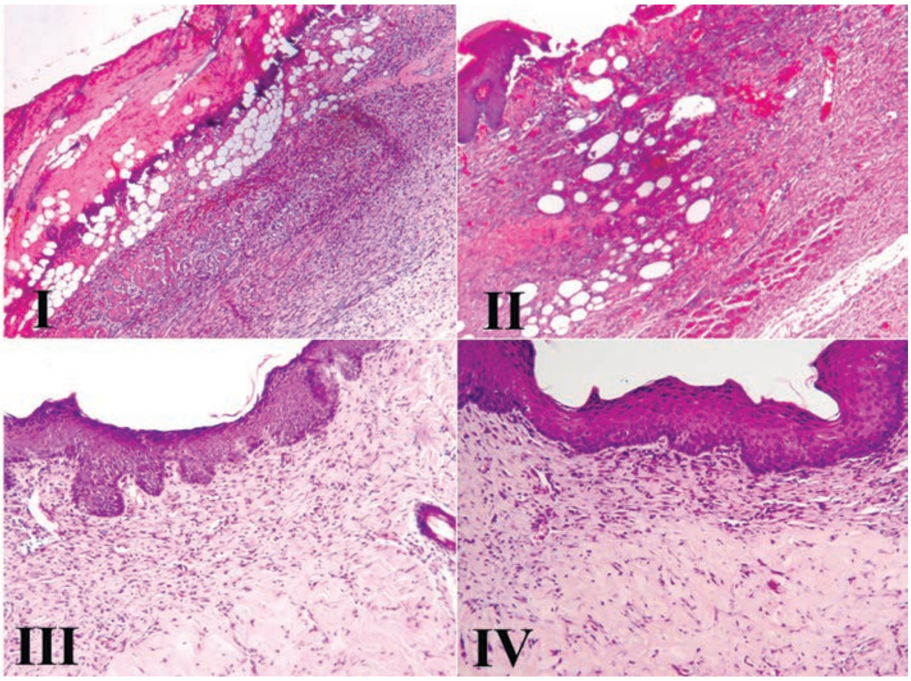

Fig. 2. Morphological structure of the skin in the experimental groups: I – CONTR, II – LAN, III – LAN+extGND, IV – LAN+extFP; hematoxylin and eosin staining; ×100

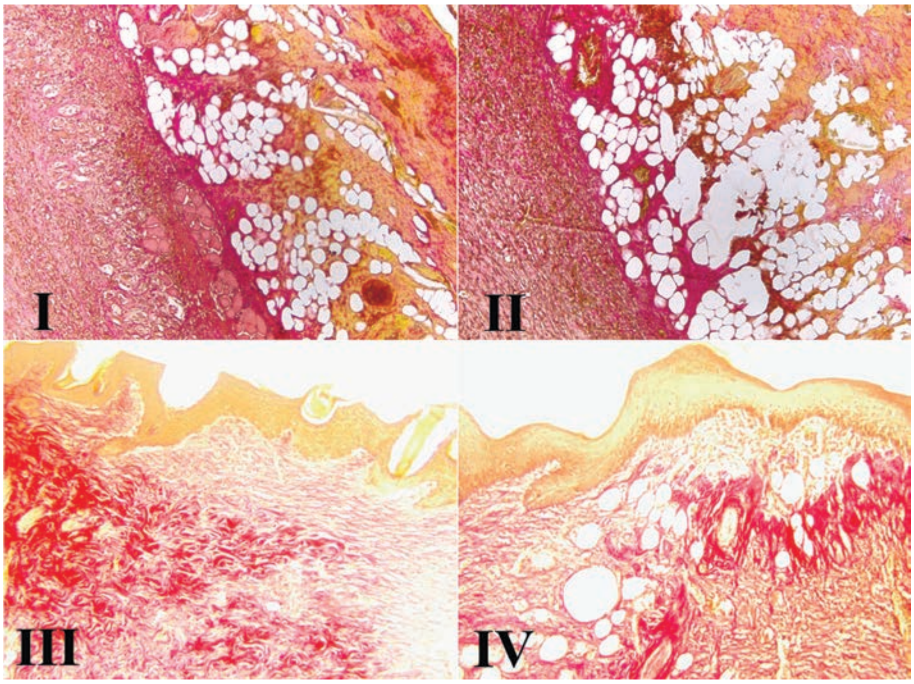

Fig. 3. Morphological structure of the skin in the experimental groups: I – CONTR, II – LAN, III – LAN+extGND, IV – LAN+extFP; Van Gieson's staining; ×100

The CONTR and LAN groups exhibited microcirculatory disfunction, such as vasodilation, leukocyte stasis and diapedesis, and vascular congestion. Both fungal extract groups showed reduced vascular reactions. The LAN+extFP group demonstrated smaller vessel area (Fig. 4, *b*). Despite a comparable vessel number (Fig. 4, *c*), the LAN+extGND group showed a decreased vessel number with leukocyte stasis and diapedesis (Fig. 4, *d*). Both groups also had a reduced number of vessels with blood stasis (Fig. 4, *e*).

Inflammation hindered scar formation in the CONTR and LAN groups, therefore it comprised mainly hematogenous cells. By contrast, in the fungal extract groups, a large number of fibroblasts were found (Fig. 4, *f*). Moreover, for the LAN+extGND group, the differences were noted compared to not only the control group, but also to the LAN group.

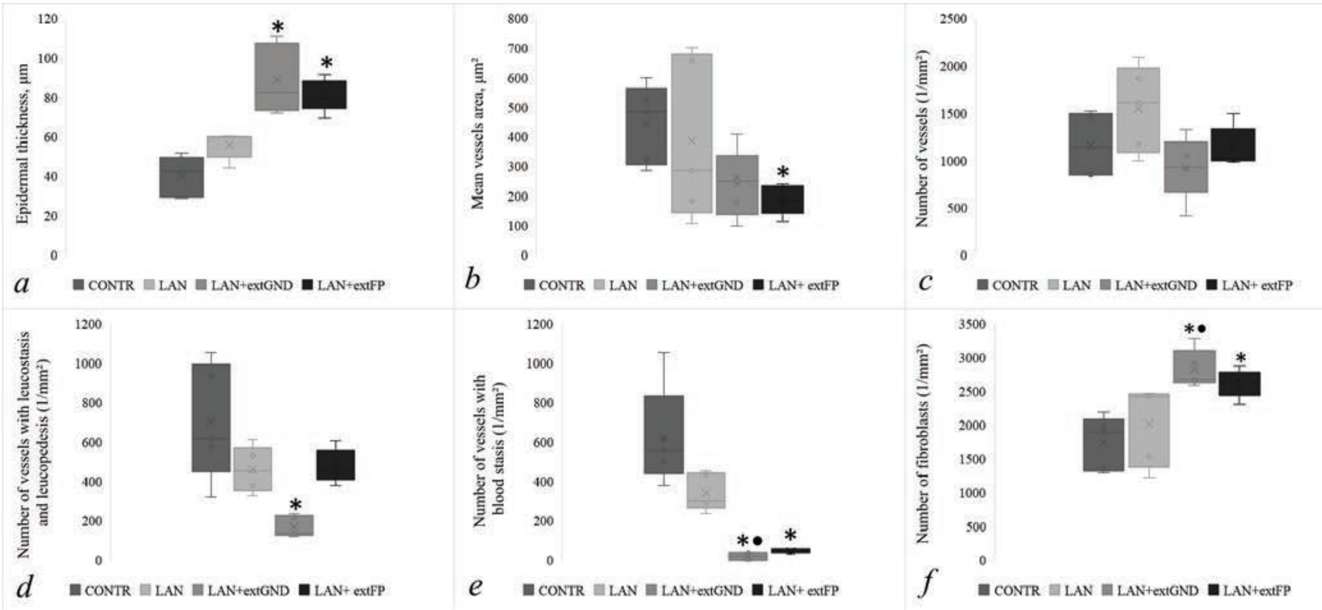

Fig. 4. Results of the morphological and quantitative assessment of the regenerated skin: * significant differences compared to the CONTR group, $p < 0.05$; ● significant differences compared to the LAN group, $p < 0.05$

DISCUSSION

Accelerated wound healing in the experimental fungal extract groups correlated with reduced exudation, improved microcirculation, and diminished inflammation, promoting faster tissue restoration and smaller wound areas. Acanthosis in the LAN+extGND group signaled active re-epithelialization, while mature collagen bundles indicated scar maturation. Nevertheless, a high vascularization degree characterized the scar as normal rather than fibrous. Reduced leukocyte stasis and diapedesis in the LAN+extGND group aligned with anti-inflammatory effects of the *G. applanatum* extract compounds.

In the LAN+extFP group, epithelialization was also pronounced, however, the fibrous component formation was not as intense. In addition, the infiltrate presence indicated a slight anti-inflammatory effect. Despite weaker anti-inflammatory effects, the *F. pinicola* extract modulated vascular reactions. It can be assumed that disparate bioactivity of extracts apparently stemed from alkaloids and saponins identified in *F. pinicola*, as well as higher flavonoid content. Both extracts likely owed their anti-inflammatory effects to phenolic compounds with antioxidant activity. Moreover, phenolic content was the same in both fungi extracts. According to numerous studies, the most potent effect of *G. applanatum* extract may be related to triterpene compounds [7].

In both fungal extract groups, a large number of fibroblasts was observed, probably associated with the inflammation and microcirculation modulation. An enhanced fibroblast count might suggest stimulating effects of fungal metabolites on cell proliferation. This is also supported by enhanced re-epithelialization, caused by proliferative activity of keratinocytes.

Greater collagen deposition under *G. applanatum* extract treatment implied a potential impact on the synthetic activity of fibroblasts. However, a detailed extract composition and effects of extractive compounds require further study.

CONCLUSION

Burn injury regeneration under fungi extract treatment proceeds through classical stages but with shortened duration and neo-tissue formation by day 14 of the experiment. Both extracts accelerated epithelialization and scar maturation probably via cell proliferation stimulation. Anti-inflammatory effects and vascular modulation were evident for *G. applanatum* and *F. pinicola*, which increased the regeneration efficiency.

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