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Research article

γ-Oryzanol: A nutrient-rich ingredient for promoting wound healing

Yi-Shan Liu [a,b,c,**], Mei Chou Lai [c], Tang-Yao Hong [d], I-Min Liu [c,*]

[a] Department of Dermatology, E-Da Hospital, I-Shou University, Kaohsiung City, 84001, Taiwan
[b] School of Chinese Medicine for Post Baccalaureate, College of Medicine, I-Shou University, Kaohsiung City, 84001, Taiwan
[c] Department of Pharmacy and Master Program, Collage of Pharmacy and Health Care, Tajen University, Pingtung County, 90741, Taiwan
[d] Department of Environmental Science and Occupational Safety and Hygiene, Graduate School of Environmental Management, Collage of Pharmacy and Health Care, Tajen University, Pingtung County, 90741, Taiwan

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ABSTRACT

γ-Oryzanol (Orz) is a powerful antioxidant found in rice bran and oil, known for its numerous health benefits. Nevertheless, its precise impact on skin wound healing remains largely unexplored. This research focuses on comprehensively examining the ability of Orz to promote tissue repair through laboratory-based and animal model experiments. The research further examines its antimicrobial activity and its role in promoting wound healing through anti-inflammatory effects. To evaluate the effect of Orz on tissue repair, scratch assays performed *in vitro* were used to analyze its impact on cellular migration. An *in vivo* experiment utilizing a rat skin wound excision model was carried out to investigate wound contraction rates and analyze histological modifications. The granulation tissue from a dead space wound model was examined to assess the levels of inflammatory mediators, free radicals, and antioxidant activity. The findings of the study demonstrated Orz improves cellular viability while facilitating the proliferation and migration of NIH-3T3 cells. It further promotes the generation of growth factors, such as fibroblast growth factor 21 and transforming growth factor-β. Moreover, Orz exhibited significant antimicrobial activity against various pathogens commonly found in wounds. An ointment with 10 % Orz showed significant effectiveness in promoting wound healing, as indicated by notable wound contraction observed on the 14th day after surgery. Histological analysis demonstrated that the application of 10 % Orz ointment resulted in remarkable tissue repair, heightened fibroblast proliferation, and improved formation of new blood vessels. The application of Orz ointment was also found to elevate interleukin-10 levels, reduce tumor necrosis factor-α levels and ED-1-immunopositive cells, and enhance antioxidant enzyme activity, thereby mitigating oxidative damage in healing tissues throughout the initial stages of tissue repair. These results indicate that Orz holds significant promise for application in the treatment of surface wounds.

1. Introduction

The skin functions as a vital shield against environmental hazards, yet wounds create vulnerable openings that undermine this

* Corresponding author.
** Corresponding author. Department of Dermatology, E-Da Hospital, I-Shou University, Kaohsiung City, 84001, Taiwan.
E-mail addresses: drjulialiu@yahoo.com.tw (Y.-S. Liu), iml@tajen.edu.tw (I.-M. Liu).

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crucial defense [1]. The complex healing process, consisting of four distinct phases-hemostasis, followed by inflammation, then proliferation, and finally remodeling—the process is designed to restore the integrity and functionality of injured tissues, with each phase being essential in repairing and enhancing the injured area [2]. Hemostasis is the initial stage, marked by vasoconstriction, platelet aggregation, and collagen binding to the basement membrane of damaged endothelial cells [3]. This stage is crucial for initiating angiogenesis and ensuring the wound site receives adequate nutrients and oxygen [4]. In the inflammatory phase, damaged cells, pathogens, and bacteria are cleared from the wound area. Neutrophils and monocytes swiftly migrate to the area of injury, resulting in the release of proteolytic enzymes, inflammatory mediators, and factors that promote cell growth and tissue repair [5]. The proliferation phase follows, characterized by re-epithelialization, the development of new vascular networks (neovascularization), and the production of connective tissue driven by the activity and movement of fibroblasts [6]. Finally, the remodeling or maturation phase aims to optimize the arrangement of collagen fibers and strengthen the tissue's tensile properties [7].

Open wounds are highly vulnerable to bacterial infections, acting as gateways for systemic infections [8]. These infected wounds not only heal at a slower rate but also tend to produce unpleasant exudates and release toxins, which can further impede cellular regeneration [8]. Although a variety of antibiotics are commonly used to treat such infections, the rapid emergence and spread of microbial resistance to these conventional treatments pose significant challenges in infection management [9]. Consequently, researchers are increasingly focusing on bioactive substances extracted from plants that have a long history of use in herbal remedies [10]. Therefore, the development of innovative medical interventions to shorten the wound healing period, which may be prolonged due to various complicating comorbidities, is essential to enhance healing outcomes.

Rice (*Oryza Sativa* L.) bran, a byproduct generated during the rice milling process to produce white rice, contains 12–18 % oil and is packed with essential vitamins and minerals [11,12]. Despite its nutritional richness, rice bran has primarily been utilized as animal feed rather than for human consumption. Notably, rice bran is abundant in γ-oryzanol (Orz), a bioactive compound known for its health-enhancing properties, including strong antioxidant activity, support for lean muscle growth, and stimulation of the hypothalamus [13]. Research indicates that γ-oryzanol can inhibit the biosynthesis of hepatic cholesterol, reduce plasma cholesterol levels, and decrease cholesterol absorption [14]. Moreover, γ-oryzanol is recognized as a safe ergogenic aid, with no significant adverse effects observed in studies conducted on both humans and animal models. It is additionally listed in the United States Food and Drug Administration database under the unique identifier UNII SST9XCL51M [15].

Scientific exploration into the wound healing properties of plants, driven by the diverse pharmacological effects of their beneficial phytoconstituents, contrasts with the relatively limited research on the application of cereals or their byproducts in wound healing [16]. Despite the well-established health-enhancing qualities of Orz, its precise influence on skin wounds remains inadequately examined. Consequently, this study aims to thoroughly explore the potential of Orz in facilitating wound healing by conducting laboratory-based and animal model experiments. Additionally, it seeks to assess its antimicrobial efficacy and anti-inflammatory effects, particularly in the treatment of chronic skin conditions.

2. Materials and methods

2.1. Antimicrobial activity

Agar-based well diffusion technique serves as a standard approach for determining the antimicrobial properties of Orz [17]. *The microorganisms tested comprised Staphylococcus aureus (ATCC 29213), Staphylococcus epidermidis (ATCC 12228), Escherichia coli (ATCC 23815), Candida albicans (ATCC 90028), Candida tropicalis (ATCC 20401) and Aspergillus niger (ATCC 1015). The microbial strains used in this study were acquired from the Bioresource Collection and Research Center at the Food Industry Research and Development Institute, situated in Hsinchu City, Taiwan. The bacterial cultures were incubated at 37°C overnight in Mueller Hinton Broth (Sigma-Aldrich, Saint Louis, MO, USA, #90922), while fungi were grown in Potato Dextrose Broth (Sigma-Aldrich, Saint Louis, MO, USA, #P6685) at 28°C for a duration of 72 h. To prepare the final inoculum,* 100 μL *of a bacterial suspension containing* 10^8 *CFU/mL and a fungal suspension with* 10^4 *spores/mL were evenly distributed onto Mueller Hinton Agar (Sigma-Aldrich, Saint Louis, MO, USA, #103872) and Potato Dextrose Agar (Sigma-Aldrich, Saint Louis, MO, USA, #70139), respectively.* A 6 mm diameter disc was impregnated with 10 μL of a 100 mg/mL solution (equivalent to 1 mg per disc) of Orz (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, #sc-295006; purity >99 %) and positioned on agar pre-inoculated with microorganisms. For bacterial testing, gentamicin (10 μg/disc, catalog #G1914) and tetracycline (10 μg/disc, catalog #T3258), both obtained from Sigma-Aldrich, Inc. (Saint Louis, MO, USA), were utilized as standard reference agents. For fungal testing, fluconazole (10 μg/disc, #F8929) and ketoconazole (10 μg/disc, #K1003), also supplied by Sigma-Aldrich, Inc. (Saint Louis, Missouri, USA), were utilized. *The minimum inhibitory concentration (MIC) was determined for microbial strains that exhibited sensitivity to Orz based on the results of the disc diffusion test. Sterile paper discs, each measuring* 6 mm *in width and impregnated with 2.5*-1000 μg *of each component* per *disc, were placed on the medium's surface.*

2.2. In vitro assessment of wound repair potential

2.2.1. Cell viability assessment

The effects of Orz on the survival rate of NIH-3T3 mouse fibroblast cells, sourced from the American Type Culture Collection, were assessed using the MTT assay, which measures cellular metabolic activity through the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide [18]. Briefly, cells were planted in a 96-well microplate at a concentration of 2×10^5 cells/mL and incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin solution. The cells were maintained at 37 °C in a humidified environment with 5 % CO_2 to support optimal growth conditions. Once cell

confluence reached approximately 80–90 %, the culture medium was replaced with a fresh maintenance solution consisting of DMEM supplemented with 0.4 % FBS. The cells were then incubated under the same conditions at 37 °C for another 24 h. Furthermore, different doses of Orz (12.5, 25, and 50 μg/mL) were applied to the cultures in growth medium and incubated for 24 h. Building on prior research, this approach demonstrates that the oryzanol-enriched fraction of rice bran mitigates hydrogen peroxide-induced toxicity in differentiated human neuroblastoma SH-SY5Y cells, as evidenced by improved cell viability and an associated upregulation of antioxidant protein expression [19]. A stock solution of 1 mmol/L Orz was prepared with dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA; Catalog #D8418). This stock solution was diluted in fresh medium to achieve the desired concentrations just before application, ensuring that the resulting concentration of dimethyl sulfoxide (DMSO) in the final solution remained below 0.5 % (v/v). Cells cultured in an equivalent volume of culture medium served as the control group for comparison. The absorbance at 490 nm was recorded using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.2.2. *In vitro* wound closure assay

An *in vitro* scratch assay was utilized to evaluate the migratory capability of cells [20]. The NIH-3T3 cells were plated in 6-well dishes at a concentration of 2×10^5 cells per milliliter and cultured in a fully supplemented medium until their confluency reached approximately 80–90 %. The cells were then maintained in a humidified incubator set at 37 °C with 5 % CO_2 for 24 h. Following incubation, a consistent scratch wound was made across the cell monolayer using a sterile 200 μL micropipette tip. To ensure the removal of detached cells and debris, the culture was rinsed with phosphate-buffered saline. The cells subjected to scratching were exposed to different concentrations of Orz (12.5, 25, and 50 μg/mL) and subsequently incubated for either 12 or 24 h. The progression of wound closure was monitored under an inverted microscope (Olympus, Tokyo, Japan), and the remaining scratch zone was quantified utilizing ImageJ (version 1.38, developed by NIH, Bethesda, MD, USA) for analysis.

2.2.3. Determination of growth factors

Following a 12-h incubation, the harvested supernatants from each well of the scratch assay was collected and combined. Alterations in fibroblast growth factor (FGF)21 (#ab223589) and transforming growth factor (TGF)-β1 (#ab119557) were analyzed using enzyme-linked immunosorbent assay (ELISA) kits purchased from Abcam Inc. (Cambridge, MA, USA), in accordance with the protocols provided by the supplier. The optical density at 450 nm in every well was measured with a SpectraMax M5 microplate reading instrument (Molecular Devices, Sunnyvale, CA, USA).

2.3. Research animals

Male Wistar rats, aged 6–8 weeks and weighing approximately 200–250 g, were obtained from the National Laboratory Animal Center located in Taipei, Taiwan. The animals were housed in a facility with a regulated temperature of 25 ± 1 °C and maintained under a 12-h light/dark cycle, with lights scheduled to turn on at 06:00 h, and provided with an unrestricted supply of food and water. The rats were allowed a 7-day acclimation period to familiarize themselves with the laboratory conditions prior to the start of the experiments. All procedures involving animals were performed in compliance with the Animal Welfare Act and adhered to the guidelines outlined in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Tajen University, under approval number IACUC 112-19, issued on January 21, 2023.

2.4. In vivo wound healing activity

2.4.1. Excision wound model

Anesthesia was administered to five groups of eight rats each using the ether-based open-mask procedure. The dorsal fur of the rats was shaved, and a 500 mm^2 full-thickness excisional wound was created by carefully removing a defined section of skin, leaving the wound exposed to the environment. Various treatments were administered topically to the wounds once daily until complete healing was achieved. These included a simple ointment, Orz ointments at concentrations of 5 % w/w and 10 % w/w, and a reference formulation containing 0.2 % w/w nitrofurazone, with each wound receiving approximately 0.20 g of the respective treatment. The group that did not receive any ointment treatment after injury was designated as the blank control group.

Following the guidelines of the British Pharmacopoeia, an ointment was formulated by combining 170 g of white soft paraffin, 10 g of hard paraffin, 10 g of cetostearyl alcohol, and 10 g of wool fat [21]. Orz ointments at concentrations of 5 % and 10 % (w/w) were prepared by blending 5 g and 10 g of Orz with 100 g of a basic ointment formulation, respectively. Ointment containing nitrofurazone (manufactured by GSK Pharmaceuticals, Bangalore, India) was utilized as a benchmark medication for evaluating the wound-healing potential of Orz ointment. The wound area was measured every other day by tracing it on graph paper marked with a millimeter grid for measurement. The extent of wound contraction was determined by calculating the percentage decrease in the wound's surface area.

2.4.2. Dead space wound model

The rats were placed under anesthesia, and a 1 cm incision was carefully performed on the dorsolumbar region of their back. Two polypropylene tubes, each measuring 0.5×2.5 cm^2, were placed in the lumbar region bilaterally, and the incisions were secured with sutures to close the wounds [22]. The animals were euthanized on days 3 and 10 post-injury. The granulation tissue surrounding the implanted tubes was carefully excised and examined to assess the concentrations of pro-inflammatory and anti-inflammatory cytokines, alongside the evaluation of free radicals and antioxidant capacity.

2.5. Assessment of inflammatory biomarkers and antioxidant potential

Three days post-creation of the dead space wound model, granulation tissue formed around the implanted tubes was harvested from each experimental group. This was carried out to evaluate the levels of cytokines that promote inflammation and those that counteract it. Seven days after the creation of the dead space wound model, the granulation tissue that developed on the implanted tubes was collected to evaluate oxidative stress markers and antioxidant properties [23]. The granulation homogenate was generated using a solution containing 0.15 mol/L of KCl and subjected to spun at 8000 rpm for 10 min using a centrifuge. The obtained supernatant, free of cells, was utilized to measure cytokines, oxidative stress, and antioxidant activity.

The ELISA kits specific for tumor necrosis factor (TNF)-α (#ab236712) and interleukin (IL)-10 (#ab100764) were obtained from Abcam Inc. (Cambridge, MA, USA), and the assays were performed according to the protocols provided by the manufacture. The cytokine levels were reported in units of pg per mg of protein.

The levels of lipid peroxidation, evaluated as a parameter for assessing oxidative stress, were quantified by measuring malondialdehyde (MDA) released during lipid peroxidation using a competitive ELISA kit (#ab238537) according to the instruction manual provided by the manufacturer (Abcam Inc., Cambridge, MA, USA). The level of MDA, expressed as nmol per mg of protein, was quantified by measuring absorbance at 450 nm with a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

The evaluation of antioxidant activity was conducted using a spectrophotometric colorimetric assay. Colorimetric assay kits of superoxide dismutase (SOD; # ab65354), glutathione peroxidase (GPx; # ab102530), and catalase (CAT; # ab83464) were purchased from Abcam Inc. (Cambridge, MA, USA) for enzymatic activity assessments. The enzymatic activity of SOD was determined by measuring absorbance at 450 nm, while GPx and CAT activities were assessed at 340 nm and 570 nm, respectively, using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Enzymatic activities were standardized and reported as specific activity, measured in units per milligram of protein. The total protein content in the samples was measured using the Bradford protein assay. To ensure the reliability of the results, all experiments were performed in triplicate.

2.6. Immunohistochemistry

In a rat excision wound model, skin tissues from the regenerated wound site were harvested on the third day post-injury. The collected samples were preserved in formalin and subsequently embedded in paraffin, and analyzed for inflammatory *macrophages* using immunohistochemical staining. To prepare the tissue sections, paraffin was removed, and the slides were rehydrated before being immersed in Tris-buffered saline (10 mM Tris-HCl, 0.85 % NaCl, pH 7.2) for thorough cleansing. In order to suppress intrinsic peroxidase activity, the specimens were immersed in a methanol-based solution with 0.3 % hydrogen peroxide. The specimens were subsequently placed at 4 °C overnight with an anti-ED1 antibody specific to macrophages and monocytes (Sigma-Aldrich, St. Louis, MO, USA; Cat. No. MAB1435) for incubation, followed by washing with TBS. To proceed with the staining process, the slides were further incubated at ambient temperature for 1 h following the application of a goat anti-mouse secondary antibody conjugated with horseradish peroxidase. Following a TBS rinse, the slides were exposed to diaminobenzidine tetrahydrochloride as a chromogenic substrate, then subjected to a hematoxylin counterstain for contrast enhancement. Color deconvolution was performed to distinguish the diaminobenzidine reaction product from the hematoxylin counterstain. The ED-1-stained areas were then analyzed in pixels with Image J 1.38 software (NIH, Bethesda, MD, USA). The quantification of ED-1 positive cells is determined by calculating the percentage of ED1 expression in relation to the unit area.

2.7. Quantitative assessment of hydroxyproline and hexosamine concentrations

On the 14th day following injury in the full-thickness skin wound rat model, the hydroxyproline concentration was assessed in skin tissue obtained from the regenerated wound site. The samples were dehydrated in a hot air oven at a controlled temperature of 60–70 °C until a stable weight was achieved. Subsequently, they were subjected to hydrolysis in sealed containers using 6 N HCl at 130 °C for a duration of 4 h. The hydrolysate was neutralized to pH 7.0 with 0.1 N KOH, followed by a 20-min chloramine-T oxidation, which was terminated through the introduction of 0.4 mol/L perchloric acid. The colorimetric reaction was induced by Ehrlich's reagent at 60 °C, and the absorbance at 557 nm was measured using a SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). To measure hexosamine levels, granulation tissues were subjected to hydrolysis in 6 N HCl at 98 °C for 8 h, followed by pH neutralization to 7 using 4 N NaOH, and subsequently adjusted with Milli-Q water. Hexosamine content was determined using a modified method by mixing the diluted solution with acetylacetone, heating it at 96 °C for 40 min, then cooling before adding 96 % ethanol and Ehrlich's reagent. After thorough homogenization and 1-h incubation at ambient temperature, hexosamine levels were quantified by measuring absorbance at 530 nm using a SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) and referencing a calibration curve.

2.8. Pathological assessment

Skin specimens were immediately placed in a buffered formaldehyde-based fixative, which was replaced every 48 h to maintain tissue integrity. Following sample processing, tissues were encased in paraffin and sectioned into ultrathin slices of approximately 3 μm thickness. Morphological features of these sections were examined following staining with hematoxylin and eosin (H&E). Microscopic observation under a light microscope was performed for qualitative evaluation. Sections were graded on a scale from 0 to *4*, as follows: 0 (<5 *%)*, *1* (5–*25 %)*, *2* (26–*50 %)*, *3* (51–*75 %)*, and *4* (>*75 %)* based on the relative degree of neutrophil infiltration, collagen

formation, fibroblast growth and new blood vessel formation, along with the full development of the epidermis to facilitate epithelialization.

2.9. Statistical assessment

The values are reported as the arithmetic mean along with the corresponding standard deviation (SD). Statistical analysis included one-way analysis of variance (ANOVA). *Statistical computations were carried out with SigmaPlot (v14.0, Systat Software Inc., San Jose, CA, USA) for data interpretation and analysis.* When necessary, post-hoc comparisons were conducted using the Dunnett test to identify the sources of significant variations. The differences were statistically significant at $p < 0.05$.

3. Results

3.1. Microbial growth suppression

γ-Oryzanol has demonstrated microbial growth suppression, affecting all tested organisms with varying degrees of efficiency (Table 1). Orz exhibited the strongest inhibitory effect against *Candida tropicalis,* with decreasing potency observed against *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. The MIC of Orz varied between 100 and 250 μg per disc. Among the tested organisms, *Candida tropicalis* exhibited the highest sensitivity, with an MIC of 100 μg per disc (Table 1).

3.2. Impact on cell viability

γ-Oryzanol demonstrated notable cell viability, with levels consistently surpassing 70 % across various concentrations (12.5–50 μg/mL) tested over a 24-h period (Fig. 1).

3.3. Effects on scratch assay-based wound healing

According to Fig. 2A, progressive cell migration into the scratched area became apparent at both the 12-h and 24-h time points. Notably, the application of Orz led to a concentration-dependent decrease in wound size, in contrast to the control group, which exhibited no exposure to Orz. Following a 24-h incubation period, a significant enhancement in wound closure was observed, which was attributed to the effects of Orz when present at 50 μg/mL (Fig. 2A). Moreover, after 12 h of conducting the scratch assay, the concentrations of both FGF21 and TGF-β1 in the culture supernatant exhibited a concentration-dependent increase with Orz treatments, as illustrated in Fig. 2B.

3.4. Impact on tissue regeneration in an excisional wound model

The findings indicated that the wound healing efficacy of the sample ointment was comparable to that of the blank control group, with no significant difference observed between the two (Table 2). However, animals treated with Orz ointment exhibited enhanced wound healing compared to both the blank control and sample ointment-treated groups (Table 2). The efficacy of Orz ointment in promoting wound healing was found to correlate with its concentration, showing particularly strong effectiveness at a 10 % concentration. Although the group receiving nitrofurazone ointment exhibited a more rapid wound healing process compared to the others, complete healing was still achieved by day 14 (Table 2).

3.5. Impact on inflammatory biomarkers in granulation tissues

By the third day following wound induction in the dead space model, no significant variation was observed in IL-10 and TNF-α levels between the blank control group and the sample ointment-treated group. However, the granulation tissue from groups treated with Orz or nitrofurazone ointments exhibited elevated levels of IL-10 compared to the blank control or groups treated with a simple

Table 1
Antimicrobial activity of tested compounds.

Microorganisms	Zone of inhibition in diameter (mm)					MIC values (μg/disc)
	Tetracycline (10 μg/disc)	Gentamicin (10 μg/disc)	Fluconazole (30 μg/disc)	Ketoconazole (10 μg/disc)	Orz (1 mg/disc)	Orz
Staphylococcus aureus	29.6 ± 0.5	30.1 ± 0.4	–	–	11.9 ± 0.7	200
Staphylococcus epidermidis	31.8 ± 0.3	31.8 ± 0.6	–	–	12.7 ± 0.5	200
Escherichia coli	21.3 ± 0.4	35.1 ± 0.5	–	–	10.3 ± 0.6	250
Candida albicans	–	–	29.9 ± 0.6	30.6 ± 0.4	9.8 ± 0.4	250
Candida tropicalis	–	–	36.1 ± 0.8	33.7 ± 0.3	13.7 ± 0.5	100

Values are mean ± SD of three replicate experiments. –: no data available.

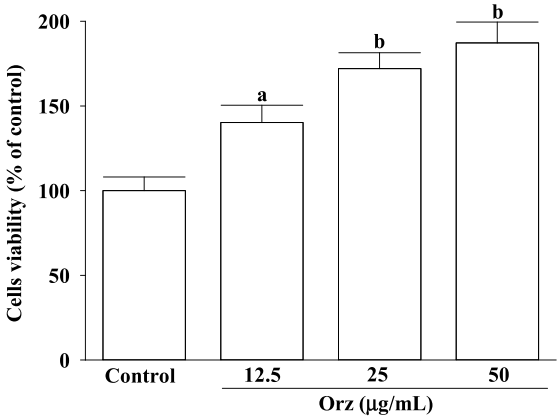

Fig. 1. Percent viability of NIH-3T3 cells treated with Orz. Data are expressed in mean ± SD of five independent experiments (n = 5) performed in triplicate. $^{a}p < 0.05$ and $^{b}p < 0.01$ compared to the untreated control group (control).

ointment (Table 3). The effectiveness of Orz ointment in enhancing IL-10 levels was especially pronounced when applied at a 10 % concentration (Table 3).

The levels of TNF-α exhibited an increase in the granulation tissue on the 3rd day post-injury in a dead space model for the blank control group and the groups treated with simple ointment, in contrast to groups receiving Orz or nitrofurazone ointment treatment (Table 3). The group treated with the 10 % (w/w) Orz ointment exhibited a significant decrease in TNF-α levels compared to those receiving the 5 % (w/w) formulation (Table 3).

3.6. Impact on lipid peroxidation and antioxidant defense in granulation tissues

Seven days after inducing a dead space wound, the granulation tissue exhibited elevated MDA levels in both the blank control group and the group treated with simple ointment. In contrast, the MDA levels were reduced in the groups receiving Orz ointment or nitrofurazone treatment (Table 4).

The enzymatic activities of SOD and GPx as well as CAT in the granulation tissue were observed to decrease 7 days after injury in the dead space wound model. This decrease was less significant in rats that received Orz or nitrofurazone ointment treatment compared to those in the blank control group or those treated with simple ointment (Table 4).

3.7. Impact on inflammatory cells

Abundant ED-1-positive macrophages was detected within the wound healing region of the excision wound rat model at 3 days post-injury, particularly in the blank control and simple ointment groups (Fig. 3). In comparison, wound samples from rats treated with either Orz or nitrofurazone ointments exhibited a significant reduction in macrophage infiltration (Fig. 3). This decrease was more substantial in the group that received 10 % (w/w) Orz ointment than in the group treated with 5 % (w/w) Orz ointment (Fig. 3).

3.8. Histological study

Histological analysis of tissues collected 14 days post-excision in rats is shown in the left panel of Fig. 4. It was observed that wounds treated with Orz or nitrofurazone ointment had better healing outcomes compared to the blank control group and those receiving a simple ointment. The tissues treated with Orz ointment showed improved fibroblast proliferation, increased formation of newly developed blood capillaries, and enhanced epithelial regeneration. Specifically, the group receiving 10 % (w/w) Orz ointment demonstrated a higher quantity of fibroblast cells and greater collagen deposition compared to those receiving 5 % (w/w) Orz ointment. On the other hand, tissues from the blank control group or those treated with simple ointment exhibited a disordered fibroblast arrangement, diminished collagen fiber deposition, and impaired blood vessel growth. The criteria used to score the distinct histopathological traits found in the recovered tissue of the test animals is illustrated in the right panel of Fig. 4.

3.9. Effects on hydroxyproline and hexosamine contents

Fourteen days after creating the excision wounds in the rat model, the rats that received Orz or nitrofurazone ointment treatment exhibited a notable rise in hydroxyproline and hexosamine levels compared to the blank control group or those who were treated with a simple ointment (Table 5). Moreover, in the Orz treatment group, the application of a 10 % (w/w) Orz ointment resulted in elevated hydroxyproline and hexosamine levels compared to the 5 % (w/w) Orz ointment (Table 5).

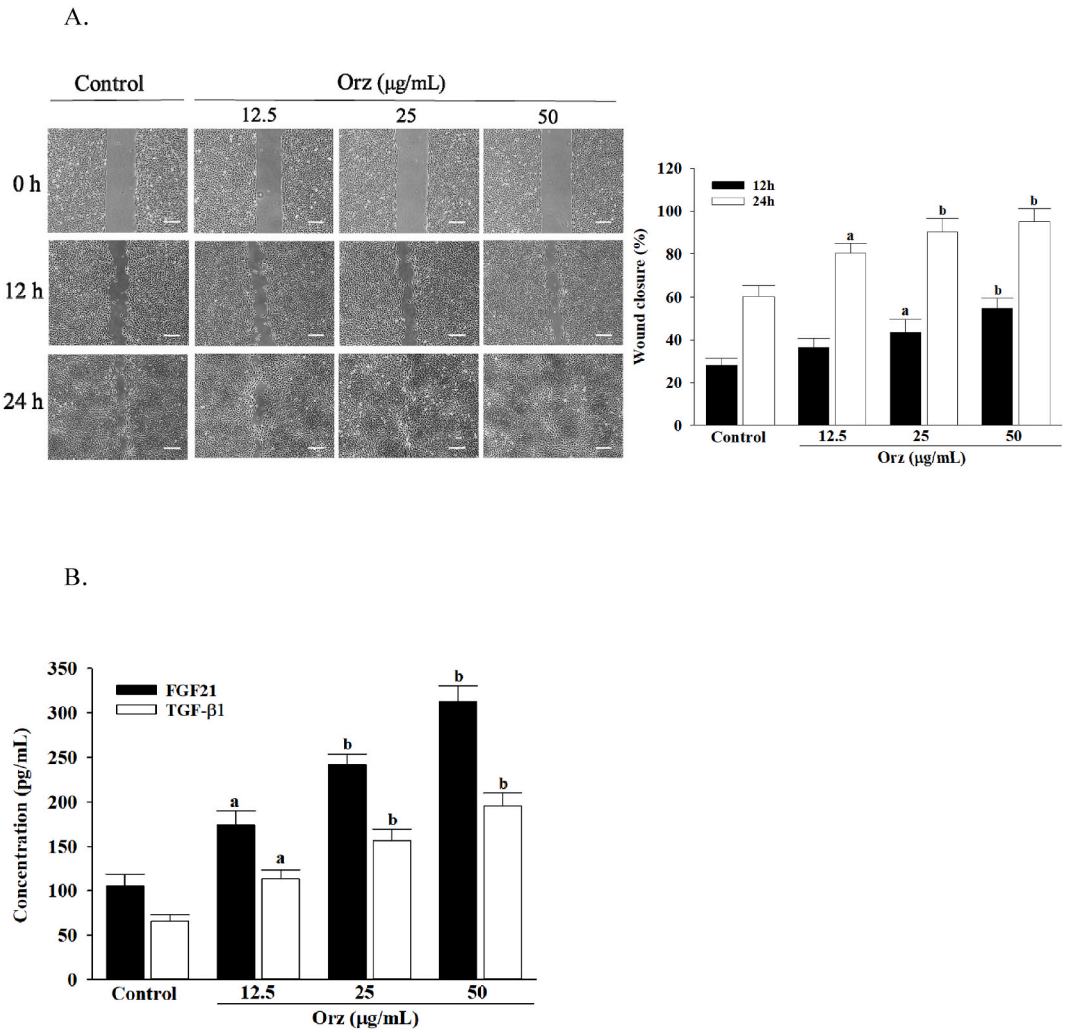

Fig. 2. Effect of Orz on the scratch closure *in vitro*. (A)The cells were observed to migrate towards the wound gap after 12 and 24 h of incubation, as seen in the representative optical images (left panel). The scale bar indicates 200 μm. The scratch closure area was then quantified using Image J 1.38 software (right panel). (B) The changes of FGF21 and TGF-β1 in the culture supernatant were measured after 12 h of the scratch assay. The data were expressed as the mean ± SD of five independent experiments (n = 5) performed in triplicate. $^{a}p < 0.05$ and $^{b}p < 0.01$ compared to the untreated control group (control).

4. Discussion

Microbial infections can seriously slow down the healing of wounds [8]. At the injury site, *Staphylococcus aureus* is the predominant Gram-positive bacterium, while *Escherichia coli* is the primary Gram-negative bacterium [24]. Furthermore, *Candida* species, yeast-type fungi, are frequently implicated in wound infections, contributing to impediments in the wound healing progression [25]. Our study has shown that Orz can be used to effectively treat various skin infections as it has shown great antimicrobial activity against all tested bacteria and fungi. The use of Orz could potentially have a significant impact in preventing the invasion of wounds by harmful microbes.

The processes of cell growth and movement play essential roles in the repair and regeneration of damaged tissues during wound healing. The scratch assay *in vitro* replicates this healing process [20]. When the integrity of the cell monolayer is compromised, neighboring cells lose direct contact. This disturbance triggers the accumulation and secretion of growth factors and cytokines at the wound site, promoting enhanced cell movement and division [26]. Our findings in this study reveal that Orz enhances cell viability and promotes fibroblast expansion and motility, accompanied by the generation of growth factors closely related to the repair cells. Among the growth factors, FGF21 has been demonstrated to facilitate the development and differentiation of connective tissue cells and vascular cells, thereby enhancing new blood vessel formation and accelerating tissue repair [27]. TGF-β1 acts as a multifunctional growth factor, playing roles in recruiting cells implicated in inflammation, bolstering tissue clearance by macrophages, fostering neovascularization, aiding in skin regeneration, and boosting collagen formation throughout the proliferative stage [28]. In our *in vitro*

Table 2
The effects of Orz and nitrofurazone ointment on wound healing in excision wounds of rat.

Post-wounding (days)	Treatments	Wound size (mm^2)	wound contraction (%)
0	Control	498.7 ± 10.1	–
	Simple ointment	498.3 ± 9.4	–
	5 % (w/w) Orz ointment	497.4 ± 7.8	–
	10 % (w/w) Orz ointment	498.6 ± 8.9	–
	0.2 % (w/w) nitrofurazone ointment	496.5 ± 9.7	–
3	Control	426.1 ± 8.4	14.5 ± 3.7
	Simple ointment	425.4 ± 7.3	14.6 ± 2.1
	5 % (w/w) Orz ointment	412.8 ± 6.2	17.3 ± 2.4
	10 % (w/w) Orz ointment	395.4 ± 8.1[a]	20.6 ± 3.1[a]
	0.2 % (w/w) nitrofurazone ointment	390.3 ± 6.1[a]	21.4 ± 2.6[a]
7	Control	315.9 ± 6.2	36.7 ± 4.3
	Simple ointment	314.3 ± 5.7	36.9 ± 4.3
	5 % (w/w) Orz ointment	295.6 ± 6.4	40.5 ± 3.8
	10 % (w/w) Orz ointment	237.4 ± 5.7[a]	52.4 ± 4.7[a]
	0.2 % (w/w) nitrofurazone ointment	225.4 ± 7.3[b]	54.6 ± 3.5[b]
14	Control	216.3 ± 8.2	55.9 ± 3.7
	Simple ointment	215.6 ± 7.1	56.7 ± 2.9
	5 % (w/w) Orz ointment	139.1 ± 5.9[b]	71.4 ± 3.2[b]
	10 % (w/w) Orz ointment	96.2 ± 6.2[b]	80.7 ± 4.1[b]
	0.2 % (w/w) nitrofurazone ointment	50.8 ± 7.4[b]	89.7 ± 3.9[b]

Values (mean ± SD) were obtained from each group of 8 animals. [a]$p < 0.05$ and [b]$p < 0.01$ compared to the values of rats treated with the blank control (control) group without any ointment treatment, respectively. –: no data available.

Table 3
Effect of Orz ointment on the levels of IL-10 and TNFα in granulation tissue on day 3 after wound creation in the dead space wound model on rat.

Treatments	IL-10 (pg/mg protein)	TNFα (pg/mg protein)
Control	22.8 ± 6.1	54.1 ± 4.8
Simple ointment	23.5 ± 4.7	53.7 ± 5.2
5 % (w/w) Orz ointment	32.7 ± 5.8	40.1 ± 4.3[a]
10 % (w/w) Orz ointment	43.2 ± 6.4[a]	32.4 ± 3.8[b]
0.2 % (w/w) nitrofurazone ointment	54.7 ± 7.1[b]	28.1 ± 3.6[b]

Values (mean ± SD) were obtained from each group of 8 animals. [a]$p < 0.05$ and [b]$p < 0.01$ compared to the values of rats treated with the blank control (control) group without any ointment treatment, respectively.

Table 4
Effect of Orz ointment on the levels of lipid peroxidation and antioxidant activity in granulation tissue on day 7 after wound creation in the dead space wound model on rat.

Treatments	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	45.9 ± 6.8	5.4 ± 1.9	6.2 ± 1.7	77.6 ± 8.9
Simple ointment	46.5 ± 6.2	5.7 ± 1.2	6.3 ± 1.1	78.1 ± 9.2
5 % (w/w) Orz ointment	28.7 ± 5.4[a]	8.3 ± 2.3	9.2 ± 1.9	118.4 ± 12.4[a]
10 % (w/w) Orz ointment	22.6 ± 7.3[a]	12.4 ± 2.8[b]	12.3 ± 2.1[a]	130.6 ± 15.6[b]
0.2 % (w/w) nitrofurazone ointment	23.7 ± 6.5[a]	11.1 ± 1.6[a]	11.6 ± 2.8[a]	127.9 ± 13.7[b]

Values (mean ± SD) were obtained from each group of 8 animals. [a] $p < 0.05$ and [b] $p < 0.01$ compared to the values of rats treated with the blank control (control) group without any ointment treatment, respectively.

wound healing model, it is plausible that Orz enhances the production of FGF21 and promotes TGF-β1-mediated wound healing through increased proliferation and migration of fibroblast cells. However, it should be emphasized that *in vitro* assays may not completely mirror the intricate wound healing process observed *in vivo*. Therefore, additional validation of this study through animal models may provide a deeper understanding of Orz's contribution to wound repair.

Incisional wounds serve as an important model for evaluating the speed of tissue contraction and epithelial regeneration [29], were employed in this study to assess the wound-healing potential of Orz ointment and its therapeutic efficacy. During the skin wound healing process, the group treated with Orz exhibited a markedly faster reduction in wound area relative to the untreated group, as observed in the results regarding wound closure. This substantial decrease in wound size attributed to Orz ointment is consistent with histopathological observations of increased epithelialization activity, angiogenesis, and collagen deposition. Collagen, which is known for providing structural integrity to tissue matrices and contributing to homeostasis and epithelialization during advanced wound healing stages [7]. Hydroxyproline is among the amino acids exclusively found in collagen [30]. Elevated levels of hydroxyproline have been observed in biochemical analyses, indicating heightened cellular growth and subsequent production of collagen [30].

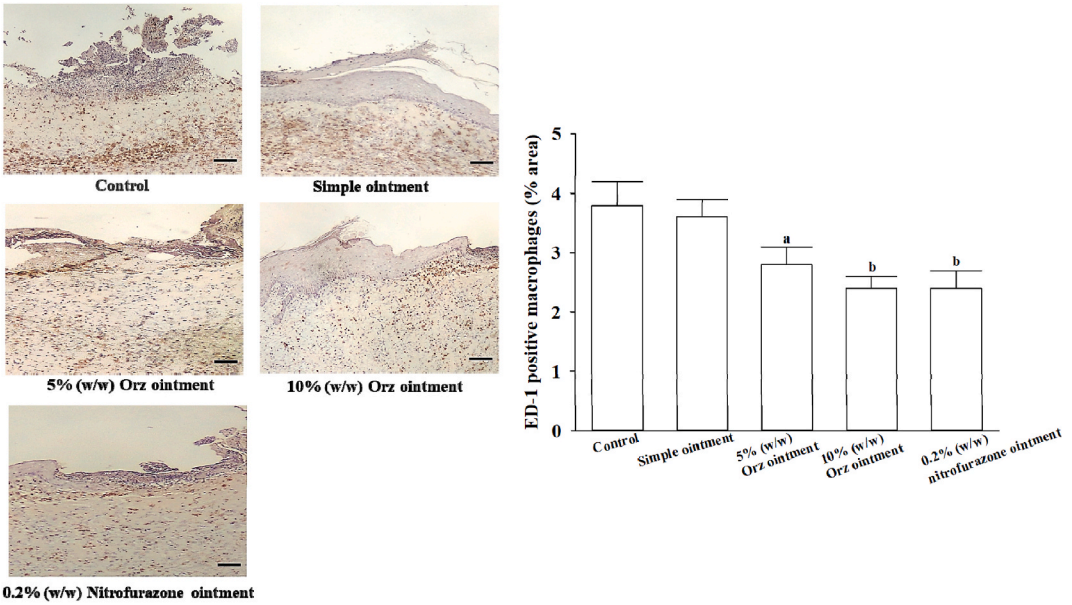

Fig. 3. Effect of Orz on inflammatory cells. Immunohistochemical staining for inflammatory macrophages (ED-1-positive) was conducted on skin samples from the healed wound area, collected on the 3rd day post-injury in an excision wound rat model (left panel). The scale bar of the representative images indicates 200 μm. The quantification of ED-1 is determined by calculating the percentage of ED-1 expression per unit area, indicated in the right panel, were expressed as the mean ± SD of 8 animals in each group (n = 8). $^{a}p < 0.05$ and $^{b}p < 0.01$ compared to the values of rats treated with the blank control (control) group without any ointment treatment, respectively.

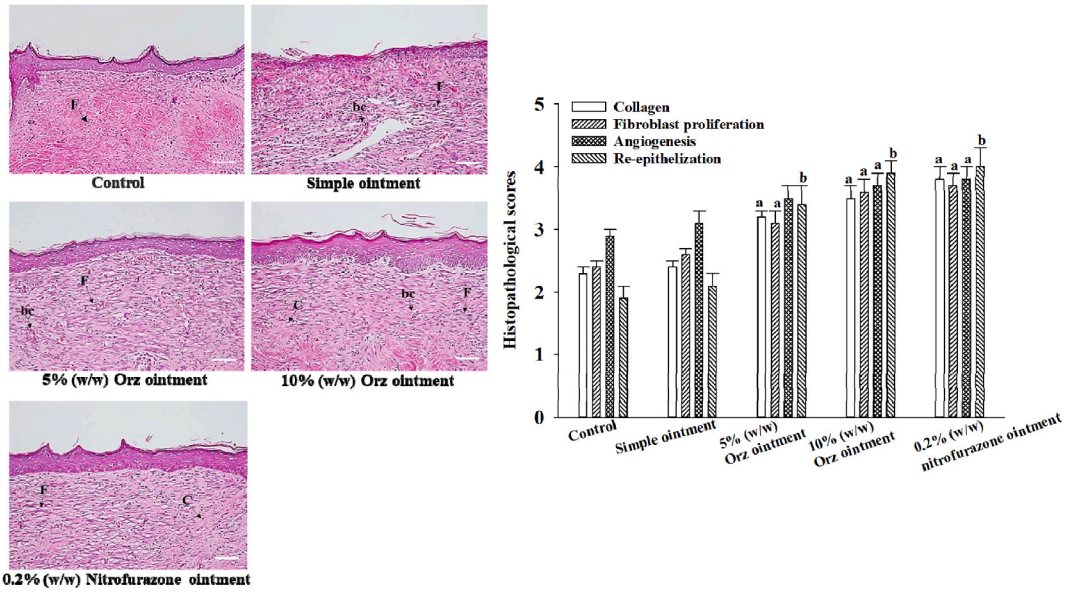

Fig. 4. Histological examination was conducted on healed wound sections stained with hematoxylin-eosin. Representative microscopic images illustrate healed wounds taken from excision wound rats treated with ointment on day 14 post-surgery (left panel). The scale bar indicates 200 μm. Abbreviations: bc for blood capillaries, C for collagen fibers, and F for fibroblasts. Histopathological scores for healed wounds, indicated in the right panel, were expressed as the mean ± SD of 8 animals in each group (n = 8). $^{a}p < 0.05$ and $^{b}p < 0.01$ compared to the values of rats treated with the blank control (control) group without any ointment treatment, respectively.

Hydroxyproline also significantly contributes to stabilizing and strengthening the triple helix structure of collagen fibers [30]. Not only does hydroxyproline contribute to collagen deposition, but an elevated hexosamine content indicates enhanced collagen molecule stabilization by strengthening the interactions between electrostatic and ionic forces [31]. Therefore, increased synthesis of hydroxyproline and hexosamine strengthens repaired tissue and affects the healing process. Research has proven that Orz ointment

Table 5
Effects of Orz ointment on the contents of hexosamine and hydroxyproline from tissue at day 14 after excision wound creation.

Treatments	Hexosamine (mg/g tissue)	Hydroxyproline (mg/g tissue)
Control	6.0 ± 0.7	44.8 ± 6.9
Simple ointment	5.9 ± 0.5	45.1 ± 6.2
5 % (w/w) Orz ointment	6.9 ± 0.4[a]	66.8 ± 9.2[a]
10 % (w/w) Orz ointment	7.8 ± 0.7[a]	78.3 ± 7.9[b]
0.2 % (w/w) nitrofurazone ointment	8.6 ± 0.6[b]	83.1 ± 8.6[b]

Values (mean ± SD) were obtained from each group of 8 animals. [a]$p < 0.05$ and [b]$p < 0.01$ compared to the values of rats treated with the blank control (control) group without any ointment treatment, respectively.

dramatically boosts hydroxyproline and hexosamine levels in excision wounds, as confirmed by histopathological analyses. The remarkable effectiveness of Orz in promoting wound healing is underscored by significant wound contraction and elevated biochemical markers in the healing tissue, providing robust support for its ethnotherapeutic claim.

The initial inflammatory phase, typically subsiding 2–3 days post-injury, marks the transition to the proliferation phase; however, excessive inflammation can impede the healing process and lead to persistent wounds with impaired recovery [26]. Maintaining an optimal equilibrium between pro-inflammatory and anti-inflammatory cytokines within the injured tissue is essential for effective healing [26]. IL-10 is a key regulator in this process, distinguished by its powerful anti-inflammatory effects and its ability to modulate TNF-α along with other inflammatory mediators [32]. Conversely, elevated TNF-α levels exacerbate inflammation and increase the risk of sepsis, making it a challenge for wound healing [26]. Our research indicates that applying Orz ointment can increase the levels of IL-10 while decreasing TNF-α levels in granulation tissue. This surge in IL-10 likely suppresses TNF-α production, thus reducing inflammation and positively affecting critical aspects of wound repairs, such as the development of granulation tissue and the organization of collagen fibers [32]. The improved rates of wound healing highlight the modulatory effects of Orz on IL-10 and TNF-α interplay and reduced persistent inflammation. Following injury, inflammatory cells like macrophages are known to release substantial amounts of pro-inflammatory cytokines during the early stages [33]. By using ED-1 accumulation as an indicator of macrophage activation [34], it showed that treatment with Orz ointment mitigated the heightened macrophage activity during the initial phase of inflammation. Overall, the infection-fighting and inflammation-reducing properties of Orz contributed significantly to accelerating the wound healing process.

An overabundance of reactive oxygen species (ROS) may trigger oxidative stress, which damages cells and interferes with the process of wound healing. Therefore, it is important to find ways to eliminate ROS as a strategy for enhancing wound healing [35]. To achieve this, it is essential to evaluate the levels of antioxidant enzymes such as SOD, GPx, and CAT in granulation tissues. These antioxidants have demonstrated their ability to neutralize free radicals and accelerate the healing of wounds [36]. Our research on antioxidant enzymes and MPO status indicates that Orz has significant antioxidant activity, which can help lower MPO levels and oxidative stress, thus mitigating inflammation and oxidative damage, which in turn can foster healing.

Despite the promising results regarding the wound-healing properties of Orz, several limitations need to be addressed. Firstly, while studies have shown that compounds such as flavonoids, triterpenoids, and tannins in plant extracts aid in wound healing [37], the specific mechanisms by which Orz components contribute to this process remain unclear. Secondly, γ-Oryzanol is a mixture of at least ten phytosteryl ferulates, with the principal components being cycloartenyl, 24-methylenecycloartanyl, campesteryl, and sitosteryl ferulates, constituting approximately 80 % of the mixture [13]. However, these components' individual or synergistic effects on wound healing have not been thoroughly investigated. This lack of specificity limits our understanding and hinders the development of targeted therapeutic applications. Moreover, comprehensive *in vivo* and clinical studies are needed to validate the effectiveness and safety of Orz in wound healing. Such studies should encompass a broader range of wound types and conditions to ensure the generalizability of the results. Furthermore, potential interactions with other wound-healing agents or medications have not been explored, which is crucial for the safe integration of Orz into existing treatment protocols. Overall, while γ-Oryzanol shows encouraging potential for wound healing, further investigation is essential to elucidate its mechanisms of action in greater detail, identify its active components, and validate its efficacy and safety across diverse clinical settings.

5. Conclusions

Our findings indicate that Orz significantly improves cell survival and stimulates the growth and movement of fibroblasts, as evidenced by scratch assays conducted *in vitro*. This is accompanied by the production of growth factors closely associated with cellular repair processes. The observed wound contraction, increased levels of hydroxyproline and hexosamine, anti-inflammatory and antioxidative effects, and moderate antimicrobial activity collectively support its reputed wound-healing properties. These multifaceted benefits suggest Orz's potential as an innovative, natural, food-based strategy for wound management. However, further in-depth studies on its mechanisms and clinical trials are necessary to validate these findings.

CRediT authorship contribution statement

Yi-Shan Liu: Writing – original draft, Investigation, Conceptualization. **Mei Chou Lai:** Methodology, Investigation, Formal analysis. **Tang-Yao Hong:** Supervision, Resources, Funding acquisition. **I-Min Liu:** Writing – review & editing, Writing – original draft,

Visualization, Resources, Project administration, Conceptualization.

Data availability statement

All data used in this study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.A. Mamun, C. Shao, P. Geng, S. Wang, J. Xiao, Recent advances in molecular mechanisms of skin wound healing and its treatments, Front. Immunol. 15 (2024) 1395479.
[2] H.N. Wilkinson, M.J. Hardman, Wound healing: cellular mechanisms and pathological outcomes, Open Biol 10 (2020) 200223.
[3] F. Scopelliti, C. Cattani, V. Dimartino, C. Mirisola, A. Cavan, Platelet derivatives and the immunomodulation of wound healing, Int. J. Mol. Sci. 23 (2022) 8370.
[4] S.J. Palmer, Nutrition essential for wound healing, Br. J. Community Nurs. 29 (2024) S32–S36.
[5] Z. Xu, B. Liang, J. Tian, J. Wu, Anti-inflammation biomaterial platforms for chronic wound healing, Biomater. Sci. 9 (2021) 4388–4409.
[6] M. Fernández-Guarino, M.L. Hernández-Bule, S. Bacci, Cellular and molecular processes in wound healing, Biomedicines 11 (2023) 2526.
[7] S.S. Mathew-Steiner, S. Roy, C.K. Sen, Collagen in wound healing, Bioengineering 8 (2021) 63.
[8] M. Mirhaj, S. Labbaf, M. Tavakoli, A.M. Seifalian, Emerging treatment strategies in wound care, Int. Wound J. 19 (2022) 1934–1954.
[9] M.A. Salam, M.Y. Al-Amin, M.T. Salam, J.S. Pawar, N. Akhter, A.A. Rabaan, M.A.A. Alqumber, Antimicrobial resistance: a growing serious threat for global public health, Healthcare 11 (2023) 1946.
[10] F. Chassagne, T. Samarakoon, G. Porras, J.T. Lyles, M. Dettweiler, L. Marquez, A.M. Salam, S. Shabih, D.R. Farrokhi, C.L. Quave, A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective, Front. Pharmacol. 11 (2021) 586548.
[11] R. Colombo, G. Moretto, M. Barberis, I. Frosi, A. Papetti, Rice byproduct compounds: from green extraction to antioxidant properties, Antioxidants 13 (2023) 35.
[12] B.L. Tan, M.E. Norhaizan, L.C. Chan, Rice bran: from waste to nutritious food ingredients, Nutrients 15 (2023) 2503.
[13] E. Ramazani, M. Akaberi, S.A. Emami, Z. Tayarani-Najaran, Biological and pharmacological effects of gamma-oryzanol: an updated review of the molecular mechanisms, Curr. Pharm. Des. 27 (2021) 2299–2316.
[14] K. Phunikhom, J. Sattayasai, S. Tiamkao, D. Gaysonsiri, A randomized, double blind clinical study to assess the effects of a gamma-oryzanol-enriched rice bran oil on lipid profile in the hypercholesterolemic patients, J. Med. Assoc. Thai. 104 (2021) 64–69.
[15] M. Sohail, A. Rakha, M.S. Butt, M.J. Iqbal, S. Rashid, Rice bran nutraceutics: a comprehensive review, Crit. Rev. Food Sci. Nutr. 57 (2017) 3771–3780.
[16] V. Mandale, A. Thomas, R. Wavhale, S. Chitlange, In-silico screening of phytoconstituents on wound healing targets-approaches and current status, Curr. Drug Discov. Technol. 19 (2022) e301121198426.
[17] T.J. Hossain, Methods for screening and evaluation of antimicrobial activity: a review of protocols, advantages, and limitations, Eur. J. Microbiol. Immunol. (Bp) 14 (2024) 97–115.
[18] E.V. Sazonova, M.S. Chesnokov, B. Zhivotovsky, G.S. Kopeina, Drug toxicity assessment: cell proliferation versus cell death, Cell Death Dis. 8 (2022) 1–11.
[19] N. Ismail, M. Ismail, M.U. Imam, N.H. Azmi, S.F. Fathy, J.B. Foo, M.F. Abu Bakar, Mechanistic basis for protection of differentiated SH-SY5Y cells by oryzanol-rich fraction against hydrogen peroxide-induced neurotoxicity, BMC Compl. Alternative Med. 14 (2014) 467.
[20] W.E. Radstake, K. Gautam, C. Van Rompay, R. Vermeesen, K. Tabury, M. Verslegers, S. Baatout, B. Baselet, Comparison of in vitro scratch wound assay experimental procedures, Biochem. Biophys. Rep. 33 (2023) 101423.
[21] Department of health and social security Scottish home and health department, Office of the British Pharmacopoeia Commission, UK, British Pharmacopoeia (BP), 1988, p. 713.
[22] A. Herman, A.P. Herman, Herbal products in postsurgical wound healing - incision, excision and dead space wound models, Planta Med. 86 (2020) 732–748.
[23] A. Sungkar, D. Widyatmoko, K.Y. Yarso, B. Wasita, The effect of duration of wound skin tissue on MDA, TNF-α, IL-6, caspase 3, VEGF levels, and granulation tissue thickness in the white rat (Rattus novergicus), Bali Med. J. 9 (2020) 918–923.
[24] M. Yin, J. Li, L. Huang, Y. Li, M. Yuan, Y. Luo, U. Armato, L. Zhang, Y. Wei, Y. Li, J. Deng, P. Wang, J. Wu, Identification of microbes in wounds using near-infrared spectroscopy, Burns 48 (2022) 791–798.
[25] E.K. White, E.A. Grice, The wound microbiome, Cold Spring Harbor Perspect. Biol. 15 (2023) a041218.
[26] K. Raziyeva, Y. Kim, Z. Zharkinbekov, K. Kassymbek, S. Jimi, A. Saparov, Immunology of acute and chronic wound healing, Biomolecules 11 (2021) 700.
[27] P. Viaña-Mendieta, M.L. Sánchez, J. Benavides, Rational selection of bioactive principles for wound healing applications: growth factors and antioxidants, Int. Wound J. 19 (2022) 100–113.
[28] W. Xiaojie, J. Banda, H. Qi, A.K. Chang, C. Bwalya, L. Chao, X. Li, Scarless wound healing: current insights from the perspectives of TGF-β, KGF-1, and KGF-2, Cytokine Growth Factor Rev. 66 (2022) 26–37.
[29] I. Pastar, O. Stojadinovic, N.C. Yin, H. Ramirez, A.G. Nusbaum, A. Sawaya, S.B. Patel, L. Khalid, R.R. Isseroff, M. Tomic-Canic, Epithelialization in wound healing: a comprehensive review, Adv. Wound Care (3) (2014) 445–464.
[30] A. Orieshyna, J.L. Puetzer, N. Amdursky, Proton transport across collagen fibrils and scaffolds: the role of hydroxyproline, Biomacromolecules 24 (2023) 4653–4662.
[31] K. Kowalewski, J.C. Russell, F.M. Heron, Collagen and hexosamine in rehealing disrupted dermal wounds of rats, Can. J. Surg. 13 (1970) 196–205.
[32] W.D. Short, M. Rae, T. Lu, B. Padon, T.J. Prajapati, F. Faruk, O.O, nd Olutoye, L. Yu, P. Bollyky, S.G. Keswani, S. Balaji, Endogenous interleukin-10 contributes to wound healing and regulates tissue repair, J. Surg. Res. 285 (2023) 26–34.
[33] A. Hassanshahi, M. Moradzad, S. Ghalamkari, M. Fadaei, A.J. Cowin, M. Hassanshahi, Macrophage-mediated inflammation in skin wound healing, Cells 11 (2022) 2953.
[34] S.K. Lin, C.Y. Hong, H.H. Chang, C.P. Chiang, C.S. Chen, J.H. Jeng, M.Y. Kuo, Immunolocalization of macrophages and transforming growth factor-beta 1 in induced rat periapical lesions, J. Endod. 26 (2000) 335–340.

[35] Y. Dong, Z. Wang, ROS-scavenging materials for skin wound healing: advancements and applications, Front. Bioeng. Biotechnol. 11 (2023) 1304835.
[36] L. Deng, C. Du, P. Song, T. Chen, S. Rui, D.G. Armstrong, W. Deng, The role of oxidative stress and antioxidants in diabetic wound healing, Oxid. Med. Cell. Longev. 2021 (2021) 8852759.
[37] S. Vitale, S. Colanero, M. Placidi, G. Di Emidio, C. Tatone, F. Amicarelli, A.M. D'Alessandro, Phytochemistry and biological activity of medicinal plants in wound healing: an overview of current research, Molecules 27 (2022) 3566.