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Evaluation of In Situ Gel Containing Pycnogenol for Cutaneous Wound Healing

Piknogenol İçeren In Situ Jelin Yara İyileşme Özelliği İçin Değerlendirilmesi

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ABSTRACT

Aim: *Pycnogenol® (PYC) are used for various medicinal purposes. The aims of the present study were to evaluate wound healing activity of PYC loaded in situ gel in mice and to investigate its antibacterial activity.*
Method: *Temperature-sensitive in situ gel containing 5% PYC was formulated by cold method using Poloxamer 188, Poloxamer 407. Blank and drug loaded in situ gel formulations were evaluated for clarity, pH, viscosity, gelation temperature, gellation capacity. The wound healing effect was tested by in vivo wound model. PYC in situ gel was administrated topically at a concentration of 5% for the 10 consecutive days after skin injury. Wound closure was measured for 10 days and at 10th day wound healing was assessed by levels of angiogenesis, granulation tissue thickness, epidermal and dermal regeneration. Its antimicrobial property was evaluated by Agar well diffusion test.*
Results: *The clarity, pH, viscosity, gellation capacity of in situ gels were found to be satisfactory.Results showed that PYC in situ gel exhibited remarkable wound healing activity with the 86.91% reduction of the wound area at the day 10 on the circular excision wound model compared to control group. Moreover PYC showed significant effect on angiogenesis, granulation tissue thickness, epidermal and dermal regeneration compared to the control group. In addition to this, PYC demonstrated antibacterial and antifungal activities. The most sensitive strains were B. cereus (23.66 mm), C. albicans (22.66 mm), and S. aueus (23 mm).*
Conclusion: *Results indicated that PYC in situ gel enhanced wound healing effectively, and so it may be developed as a an effective agent to improve wound healing in future studies to be performed.*

Keywords: *Wound healing, pycnogenol, in situ gel, mice*

ÖZ

Amaç: *Pycnogenol® (PYC) çeşitli tıbbi amaçlar için kullanılmaktadır. Bu çalışmanın amacı, farelerde PYC yüklü in situ jelin yara iyileşme aktivitesini değerlendirmek ve antibakteriyel aktivitesini araştırmaktır.*
Yöntem: *%5 PYC içeren sıcaklığa duyarlı in situ jel, Poloksamer 188, Poloxamer 407 kullanılarak soğuk yöntemle formüle edilmiştir. Boş ve etkin madde yüklü in situ jel formülasyonları, berraklık, pH, viskozite, jelasyon sıcaklığı, jelleşme kapasitesi açısından değerlendirilmiştir. PYC in situ jelin yara iyileştirici etkisi in vivo yara modeli ile test edildi. PYC in situ jel 10 gün boyunca topikal olarak %5'lik bir konsantrasyonda uygulandı. Yara kapanması 10 gün boyunca ölçüldü ve 10. günde yara iyileşmesi anjiyogenez, granülasyon dokusu kalınlığı, epidermal ve dermal rejenerasyon seviyeleri ile değerlendirildi. Antimikrobiyal özellik Agar well difüzyon testi ile değerlendirildi.*
Bulgular: *In situ jellerin berraklık, pH, viskozite, jelleşme kapasitesi uygun özellikte bulunmuştur. PYC in situ jelin, yara modelinde kontrol grubuna kıyasla 10. günde yara alanında %86.91 küçülme ile belirgin yara iyileşme aktivitesine sahip olduğunu görüldü. Ayrıca PYC in situ jel, kontrol grubuna göre anjiyogenez, granülasyon dokusu kalınlığı, epidermal ve dermal rejenerasyon üzerinde anlamlı bir etki göstermiştir. Buna ek olarak, PYC antibakteriyel ve antifungal aktivite göstermiştir. En hassas suşlar B. cereus (23.66 mm), C. albicans (22.66 mm), S. aueus (23 mm) olarak belirlendi.*
Sonuç: *PYC içeren in situ jelin etkili bir şekilde yara iyileşmesini arttırdığı görülmüş ve yapılacak ileri çalışmalarla yara iyileşmesinde etkili bir ajan olarak geliştirilebileceği düşünülmektedir.*

Anahtar kelimeler: *yara iyileşme, piknogenol, in situ jel, fare*

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INTRODUCTION

A wound is one of the most complex processes affecting the function and integrity of the skin and can affect the deeper underlying tissues resulting in infection and inflammation[1]. Topical applications of natural medicinal products and plant extracts are becoming a promising choice for the wound healing for a few decades[2]. Pycnogenol® (PYC) is the standardized extract of the *Pinus pinaster* barks (French maritime pine)[3]. It is comprised of flavonoids, glycosides, cinnamic acids, phenolic acids, mainly procyanidins (65-75%), and taxifolin[4]. Nowadays, PYC (patented by Horphag Research, Ltd. Switzerland) expresses a certain mixture of procyanidins extracted from the bark of *P. pinaster*[5]. It has been demonstrated by in vivo and in vitro experiments that PYC possesses antimicrobial, antioxidant, anti-inflammatory activities[6].

As a local drug delivery system, in situ gel-forming system has generated a great interest in dermal traumas[7]. These systems are aqueous solutions however after administration they transform to gel-form under physiological conditions. There are varied mechanisms such as ionic cross-linkage, temperature modulation, and pH change that bring about in situ gel formation[8]. In situ gelling system will cover all the limitations of the conventional dosage forms and aids in formulation of an easily dropped preparations[9]. Thus, these in situ gels possess advantages of both gels and solutions, they may develop the retention time of drug as well the formulations, and provide ease of application and accuracy[10]. The polymers such as Poloxamer 188 (P188) and 407 (P407) are generally utilized for preparation of thermoreversible gels. Dermal carriers of thermosensitive formulations are being altered to obtain good bioavailability and compliance[11].

In this study, PYC loaded in situ gel formulation was prepared as a wound healing agent to improve healing and inhibit inflammation. A blend of poloxamers 188 and 407 was used for preparations of gels with wound healing properties. The aim of the present study was to prepare and characterize blank and PYC loaded in situ gels and to evaluate wound healing properties of PYC loaded in situ gel using Balb-c mice.

MATERIALS AND METHODS

Materials

In this work distilled water was used. Poloxamer 188 and 407 were the kind gift from BASF (Turkey). Pycnogenol® was supplied by Solgar (Turkey) and Madecassol® cream was supplied by Bayer (Turkey). For microbiological studies, plates were obtained from LP, Italiana, the standard of McFarland (ref. 70900) from Biomerieux, Mueller-Hinton II Agar from Sigma (Germany), and Nutrient Agar from Merck (Germany).

Preparation of in situ gels

Poloxamer analogs were used as the gelling agents, and the formulations were developed by using a cold method[8,12]. Poloxamer 407 and Poloxamer 188 were dispersed in cold water (5±1°C) (approximately 2 hours). The polymer solution was kept at 5±1°C for 24 hours. After detection of the optimum blank gel compositions, PYC (5%) was added in poloxamer solutions with constant stirring till they were entirely dissolved. The formulation was kept at 5±1°C for 48 hours to see a possible phase separation.

Determination of sol-gel temperature

Formulation (20 g) was filled into a glass beaker. A thermometer was immersed into the polymer solution and the sample was heated at the rate of 2±0.5°C/min at 200 rpm. The temperature at which the bar stopped moving was saved as the gelation temperature. Optimum poloxamer ratios were determined and selected with a sol-gel temperature of 32-34°C which is the skin surface temperature. The experiments were repeated four times[8].

Characterization of PYC loaded in situ gels

Optimum in situ gel was selected according to the gelling temperature of the formulation. The prepared optimum PYC loaded in situ gels was evaluated as for its clarity, pH, gelling capacity and viscosity. The experiments were repeated four times[8].

Clarity of formulations

The clarity of in situ gels was determined by optical check under dark color background, and it was scaled as follows: turbid, +; clear, ++; and very clear, +++[8].

Gelling capacity

The gelling capacity of the developed in situ gel was detected by droping the in situ gel on a at 32-34°C glass surface and its gelling capacity was visually determined. It was graded as follows: +; gel after few minutes dissolves rapidly, ++; immediate gelation remains for few mins, +++; immediate gelation remains for nearly an hour[8].

Measurement of pH

The pH of the formulation was detected by a digital pH-meter. Measurements were performed four times and an average of these measurements was accepted as the pH of the in situ gels[13].

Determination of viscosity

The viscosity of the in situ gel formulations was performed with a digital viscometer (Brookfield, USA). The formulations were kept in a tube. The formulations were performed with 50 rpm at 32±2°C[14].

Spreadability of formulations

To detect spreadability blank and PYC formulations 10 cm x 10 cm glass horizontal plates were used. First of all, formulations (0.1 g) were weighted and these formulations were transferred to a plate. The temperature of the glass plate was arranged as 32±2°C and the plate was compressed under another plate. Thus, the formulation was spread out between the plates. Sixty seconds later, the plate was removed and the diameter of the spread area (cm) was measured. The experiment was repeated three times[14].

In vitro antimicrobial studies

The antimicrobial activity of PYC and blank in situ gel was detected by the agar well diffusion technique against *Bacillus cereus* (ATCC 7064), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), and *Pseudomonas aeruginosa* (ATCC 27853)[15]. Organisms were incubated at 37°C for 18 h on nutrient agar. Active cultures were adjusted to 0.5 McFarland with a sterile saline solution (0.85%). One hundred microlitres of suspension spread onto Mueller Hinton II Agar and dried, and plates drilled with a sterile cork-borer were filled with 6 mg of PYC, then they were incubated for 24-48 h at 37±2°C. Zone diameters of PYC for each isolate were masured with a ruler[16]. The experiments were repeated three times.

Laboratory Animals

Balb-c mice (24-28g) were fed ad libitum with water and food under laboratory conditions and kept in standard cages (23±1°C and 12h/12h dark-light cycle). The animal experiment was performed according to the local ethics committee regulations (Istanbul Medipol University, laboratory animals ethics comittee, Date. 20.06.2018, No:20.06.18-35).

Experimental groups and excisional wound model

32 animals were divided into 4 groups (n=8) as follows;

1: Control group (CG) (untreated group)
2: Blank in situ gel group (BG) (vehicle group)
3: PYC (5%) in situ gel group (PG) group
4: Madecassol group (MG)

All mice were anesthetized with ketamine-xylazine (10 mg/kg-80 mg/kg) and the back of each animal was depilated and washed with the solution of povidone-iodine. The two excisional wounds (5 mm diameter per lesion) were created on the shaved area by the punch biopsy. The standard drug and the formulations were topically applied once a day for 10 days.

Wound area measurement

In order to evaluate contraction of the wound, photographs of each wound were taken at days 0, 2, 4, 6, 8 and 10 (Canon Inc., Japan) with an internal scale. Camera lens was posed vertically to wounds. Wound areas were computed by an image analysis program (Image J., NIH, MD, USA)15. The healing effect of PYC was calculated as follows:

% Wound contraction=(Current Wound Area / Wound Area at the beginning) × 100

Histology

The animals were sacrificed and the wound tissues were removed on day[10]. The samples were fixed in 10% formalin, for one day and then embedded in paraffin, cut into 5 µm-thick pslices, and dyed with hematoxylin & eosine for scoring under light microscopy. The histological evaluations of wound healing process of each group were graded according a score system ranged between 1-4 points described by Galeano et al. (2006). Scoring of epidermal and dermal regeneration was performed as follows: 1=poor epidermal conformation (more than 60% of the tissue), 2=incomplete epidermal conformation (more than 40%), 3=moderate epithelial proliferation (more than 60%), 4=complete epidermal remodeling (more than 80%). Scoring of the thickness of the granulation tissue was performed as follows: 1=for thin granulation tissue layer, 2=for moderate granulation tissue layer, 3=for thick granulation tissue layer and 4=for very thick granulation tissue layer. Solely mature vessels were numbered and detected by the existence of erythrocytes in the lumen to evaluate angiogenesis. The absence/presence of hemorrhage, edema, thrombosis, congestion, and intervascular/intravascular fibrin formation were etermined to separate poorly and well-formed capillaries as follows: 1 = 1−2 vessels per site (thrombosis, hemorrhage, edema, occasional congestion), 2 = 3−4 vessels per site (few newly formed capillary vessels, moderate hemorrhage and edema, occasional congestion, intravascular fibrin deposition and absence of thrombosis), 3 = 3−4 vessels per site (newly formed capillary vessels), 4 = More than 7 vessels per site (newly formed and normal appearing capillary vessels)[17-19].

Statistical analysis

Outcomes were given as mean±standard error of mean. Calculations, statistical studies, and graphs were done utilizing by GraphPad Prism 7.0. One-way ANOVA followed by Dunnett's tests were performed to find statistical significance. P<0.05 was considered significant.

RESULTS

Preparation and characterization of PYC loaded in situ gel formulations

Temperature sensitive formulations were successfully developed by the cold technique with poloxamers. Blank/in situ gel ratio characterization of the new drug carriers are major issues to be considered in the formulation developing part. The physicochemical characterization parameters of blank and PYC in situ gels are reported in Table 1. The clarity, viscosity and gelling capacity of all the formulations were found to be satisfactory, as shown in Table 1. The pH of the developed gels ranged between 5.32 and 7.669. The pH of the in situ gels was appropriate for the dermal application.

Table 1. Clarity, gelling temperature, pH, gelling capacity, viscosity, spreadability of blank and PYC in situ gels. The data are presented as the mean ± standard deviation (SD).

Formulations	Blank in situ gel	PYC in situ gel
Clarity	+++	++
Gelling temperature (OC)	32.89±0.38	32.50±0.03
pH	7.67±0.05	5.32±0.01
Viscosity (cP)	413.77±2.50	415.13±3.18
Gelling capacity	+++	+++
Spreadability (cm)	2.20±0.17	2.31±0.15

In vitro antimicrobial studies

The antimicrobial activity of PYC was performed against two Gram-negative bacteria species (*P. aeruginosa* and *E. coli*) and two Gram-positive bacteria species (*B. cereus* and *S. aureus*) and one fungus (*C. albicans*). The antimicrobial results of PYC are reported in Table 2. Agar well diffusion test results revealed that PYC showed a good inhibition zone with the

Table 2: Agar well diffusion test results of PYC (zone mm). The data are presented as the mean ± standard deviation (SD).

Microorganism	Zone (mm)
Escherchia coli	17±1
Bacillus cereus	23.66±0.57
Candida albicans	22.66±0.57
Staphylococcus aureus	23±1
Pseudomonas aeruginosa	19±0.57

strongest value against *B. cereus* (23.66±0.57 mm), *C. albicans* (22.66±0.57 mm), *S. aureus* (23±1 mm). Blank in situ gel was also examined and no zone inhibition (0 mm) was found against all species.

Macroscopic wound healing and wound contraction

To investigate the effects of the PYC containing in situ gel on wound healing, excisional wounds were opened on the backs of mice and all wounds were treated for 10 days. The daily behaviors (food intake, activity, e.g.) of mice were observed as normal.

The macroscopic image of the wound areas on days 0, 2, 4, 6, 8, and 10 are presented in Figure 1. Crust formations were observed on the skin after several days. A residual lesion was felt on the skin after the crust fell off. It was found out that PG and BG treatments did not get irritated the skin. Furthermore, on the 10th day, wound regions which were treated with MG and PG were smooth and its appearance was close to the normal skin color.

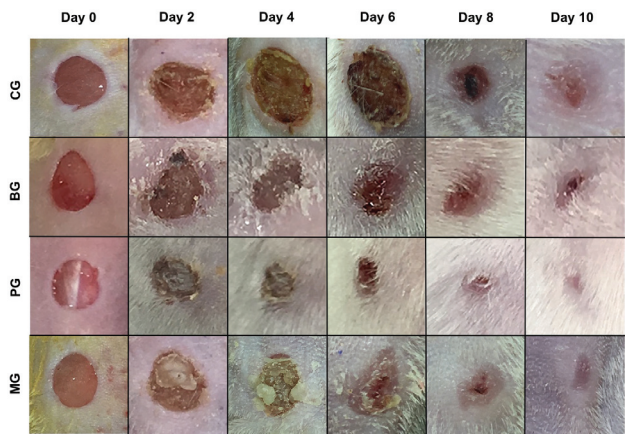

Figure 1. Macroscopic changes of the wounds healing taken for the different groups on the 0th, 2nd, 4th, 6th, 8th, and 10th post wounding day. CG were untreated (negative control); BG were treated with blank in situ gel; PG were treated with PYC in situ gel; MG were treated with "Madecassol cream®" (positive control).

The wound contraction ratio was assessed as the percentage of reduction in wound sizes on days 2, 4, 6, 8, and 10 after wounding. As it is illustrated in Figure 2, the applied group with PG showed a significant progression in wound healing on the day 4 (P<0.01), 6 (P<0.01), 8 (P<0.01) compared to the untreated group. The percentage of wound area ranged from 109.95% to 26.4% in the period from 2 to 10 days in the control group. The percentage of the wound area in mice treated with BG ranged from 118.22% to 23.45% in the period of 2-10 days. The percentage of the wound area in mice treated with MG ranged from 94.86% to 15.02% in the period of 2 to 10 days. The percentage of the wound area in mice treated with PG ranged from 96.94% to 13.09% in the period of 2 to 10 days. Compared to the control group, MG and PG recovered quickly and the wound area rapidly decreased in size by the sixth day. After 10 days, MG and PG were almost fully healed.

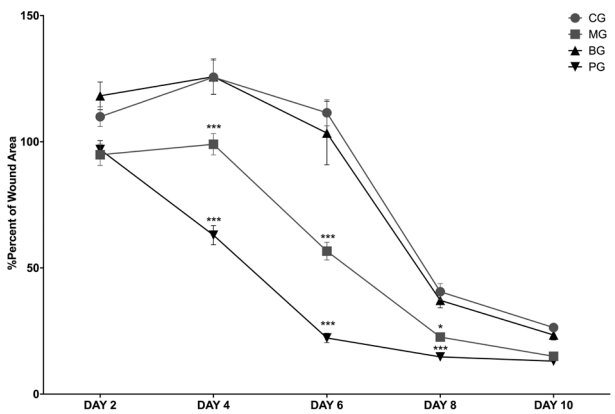

Figure 2. Effects of PYC in situ gel on wound's evolution. Healing percentage of scar tissue surface area in control (CG), blank in situ gel (BG), PYC in situ gel (PG) and madecassol groups (MG). Each data group represents the mean±SEM of eight mice. Statistically significant as compared to control; P<0.05(*), P<0.01(**), P<0.001(***).

Histology of wound healing

The microscopic photos taken during the histological examination of the wound tissues on the 10th day are given in Figure 3. Histopathological examination of wound tissues on the 10th day is illustrated in Figure 4.

As stated in the 'Results' section , it was determined that MG and PG had a significant effect compared to the control group (Figure 3-4). Moreover, it was observed that thicker granulation tissue was formed in the MG (P<0.01) and PG (P<0.01) groups after treatment in comparison to the untreated group (Figure 3-4). According to the histological evaluation results, more blood vessel formation was detected

on MG (P<0.001) and PG (P<0.001) in comparison to the untreated group. Also, PG provides new blood vessel formation comparable to MG (Figure 3-4). It has been also revealed that there were significantly higher percentages of dermal and epidermal regeneration with the MG (P<0.001) and PG (P<0.001) in comparison to the untreated group (Figure 3-4).

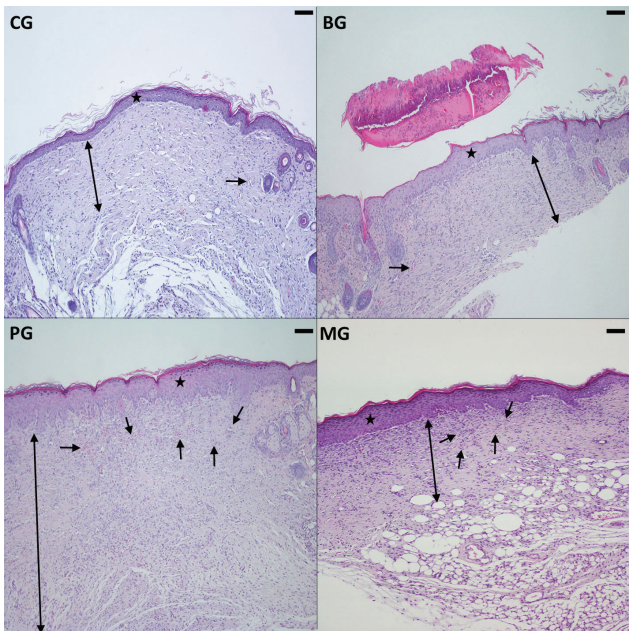

Figure 3. Histopathological view of injured tissues of the control (CG), blank in situ gel (BG), PYC in situ gel (PG) and madecassol groups (MG) on 10th day after wound incision (Hemotoxylen and eosin (H&E) (original magnificationX10). The scale bars represent 100 µm for figure. *: Epidermal regeneration, →: Angiogenesis, blood vessels, ↔: Granulation formation.

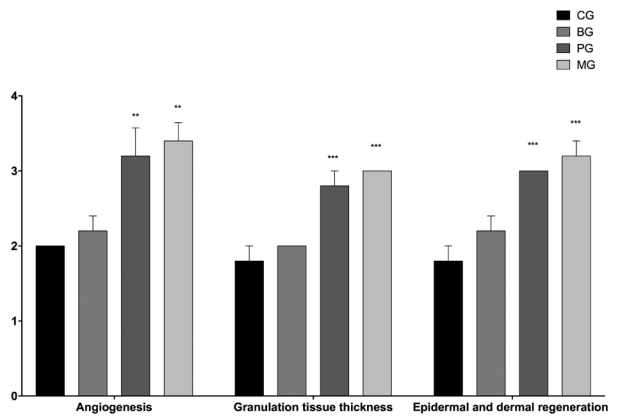

Figure 4. Microscopic examination of granulation tissue thickness, anjiogenesis and epidermal-dermal regeneration on control (CG), blank in situ gel (BG), PYC in situ gel (PG) and madecassol groups (MG) by histological wound healing scores among. Statistically significant as compared to control; P<0.05(*), P<0.01(**), P<0.001(***). Values are presented as the mean±SEM.

DISCUSSION

Plants have been traditionally utilized to improve wound healing as topical formulations. These medical plants have been determined to be very useful in wound care, promoting the rate of wound healing without discomfort to the patient, and with minimal pain, and scarring[20]. *P. pinaster* bark extract contains a mix of a large number of substances that show some activities with its antitumor, antioxidant, antimicrobial, antiatherogenic, anti-inflammatory, and antiviral properties[21].

As an effective antioxidant agent, PYC protects endogenous GSH and vitamin E and effects functional and structural characteristics of important enzymes and other cellular antioxidant networks[22]. Due to having a potent antioxidant capacity, PYC is consumed extensively as a dietary food supplement[3]. PYC contains proanthocyanidins that have been shown to exert photoprotective, antimicrobial, antioxidant, anti-inflammatory, and anti-carcinogenic effects in experimental and in vitro studies. Proanthocyanidins have a selective affinity for elastin and collagen and prevent enzymatic hydrolysis via matrix metalloproteinases in vitro. In vivo studies have shown these beneficial effects can aid the wound healing process[6].

The wound healing is a dynamic, intricate and highly regulated course including various physiological phases such as hemostasis, inflammation, proliferation, and remodeling. The advantage of antioxidant compounds that display free radical scavenging activity in wound healing is significant. Phagocytosis, caused by eosinophils, neutrophils, macrophages, monocytes, in the inflammation phase, leads to a situation known as oxidative burst, in which the depletion of O_2 significantly increases. Antioxidant compounds prevent cell damage and inhibit lipid peroxidation and increase collagen fibrillary endurance[21]. The capability of PYC to decrease oxidized ascorbate will

possibly expand the efficiency of the vitamin in the lesion area, reinforcing collagen composition. The evident binding of PYC components to elastin and collagen and their remarkable inhibitory activity on matrix metalloproteinases are likely to represent an important objective in wound healing. PYC will possibly also support the first, i.e. the inflammatory, phase of the wound healing process. Pending that stage, monocytes and neutrophils at the lesion area carry out varied tasks, such as the removal of microbes to avoid infection of the wound[3].

Dogan et al. reported that PYC and silver sulfadiazine showed equal effectiveness in lowering acute and chronic inflammation scores on days 7 and 21 compared to the untreated controls in diabetic rat wounds. Furthermore, collagen deposition and neovascularization scores were higher in wounds treated with PYC than silver sulfadiazine treated wounds[6]. According to Blazsó et al. PYC (1%) gel formulation considerably reduced the wound healing period, by 1.6 days in comparison with the blank gel group. The PYC (2%) gel treatment reduced the healing period by almost 3 days, while PYC (5%) gel further accelerated the wound healing[3].

Increased pathogenic bacteria in the wound vicinity is another component in retarded wound healing. Antiseptic agents are used in the treatment of some open wounds to kill or suppress microorganisms[6]. On the other hand, PYC possesses bacteriostatic effectiveness against a wide range of Gram-negative and positive bacteria, as well as *C. albicans*, with minimum inhibitory concentrations of 20-100 µg/mL[3].

All data obtained from the antimicrobial study showed a remarkable effect against Gram-negative and positive bacteria like staphylococcus, bacillus, and the pathogens that are commonly related to dermal infections. Hereby, it can be concluded that the antimicrobial efficiency of the PYC could provide a convenient surrounding for wound healing by preventing and managing wound infections.

Wound dressings made of biocompatible and biodegradable polymers have been used to speed the treatment of topical wound healing in the past decades. Among many wound dressings, the usage of hydrogel as a topical drug carrier in skin injury has achieved tremendous attention because the hydrogel could produce an optimum hydration for wound healing[23]. In situ formulations that assume the shape of wound deformity would be more attractive because the thermo-sensitive formulation is a free-flowing sol at 25°C, and above applied into the wound area, it will fill the injury without wrinkling or fluting[24-26]. Hence, these drug carriers have been excessively examined as wound dressing due to their advantages[24-26].

Poloxamers are triblock copolymers of polyethylene oxide and polypropylene oxide[27]. A mixture of other materials such as Poloxamer 188 or bioadhesive polymers encourages the action of Poloxamer 407 by optimizing sol-gel changeover temperature or promoting its mucoadhesive properties[8,28]. Poloxamer hydrogels are low viscosity solutions at 25°C, but they are gels at physiological temperature, making them optimum thermo-reversible polymers for dermal applications. They are also biodegradable, non-toxic, and stable[29]. Hence, a poloxamer-based thermosensitive hydrogel is a very ideal carrier for dermal treatments.

To find out an appropriate gelation temperature, the poloxamer-based gel is usually composed of two poloxamers as poloxamer 407 and poloxamer 188[30]. In this study, to find out optimum gelling temperature (32±2°C), the poloxamer 407 and poloxamer 188 were mixed at various concentrations by using the cold method. This technique with the slow participation of polymer in cold water with stir[31].

Blank in situ gel contains poloxamer 407 (20%) and poloxamer 188 (18%). Optimum drug loaded composition which contains poloxamer 407 (19%), poloxamer 188 (5%) and PYC (5%) is characterized based on its pH, clarity, and gelling capacity. Physicochemical characterization of in situ gel formulations is an important subject to be considered in the formulation

part, especially those intended for dermal application. Gelation temperature of prepared in situ gel formulations with and without PYC was changed from 32.5±0.03 to 32.888±0.379. This indicates that the formulations can be converted to gel form when they are applied on the skin surface[32]. All formulations had a clear appearance on visual inspection. The pH of a dermal formulation is critical for patient conformity. The pH of the gels was found ito range between 5.32 and 7.669. Ideally, dermal formulations should possess pH in the range of 5-6, for minimizing the discomfort of patient or skin irritation due to acidic pH and microbial growth on the skin because of basic pH[14]. This fact reveals the non-irritant characteristic of the formulation in the skin. The outcomes of the characterization analysis indicate the development of successful PYC loaded in situ gel formulation with optimum characteristics.

The determination of the wound closure rate is beneficial for scoring the progress of wound healing[15]. It can be determined that there was a remarkable decline in the rate of wound closure in PYC group compared with the control group.

Angiogenesis (neovascularization) is a complicated phase including membrane disintegration, endothelial cell reproduction and migration, and the forming of the new basement membrane from endothelial cells leading to the production of new capillaries from existent blood vessels[33]. In addition to this, enhancement of the epidermal and dermal regeneration is one of the main purposes of the wound healing. Moreover, thickness of granulation tissue is a critical parameter for objective evaluation of wound treatment[15]. In this study, it was found that the PYC in situ gel has a healing potential as revealed in the treatment group regarding epithelialization, angiogenesis, and granulation tissue thickness.

In conclusion, the present study can open up a window for dermal application of in situ gels loaded with Pycnogenol®, that will become a better alternative to conventional dermal creams in wound healing.

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REFERENCES

1. Basha M, AbouSamra MM, Awad GA, Mansy SS. A potential antibacterial wound dressing of cefadroxil chitosan nanoparticles in situ gel: Fabrication, in vitro optimization and in vivo evaluation. Int J Pharm. 2018;544:129-40. https://doi.org/10.1016/j.ijpharm.2018.04.021
2. Luo P, Li X, Ye Y, Shu X, Gong J, Wang J. Castanea mollissima shell prevents an over expression of inflammatory response and accelerates the dermal wound healing. J Ethnopharmacol. 2018;220:9-15. https://doi.org/10.1016/j.jep.2018.03.020
3. Blazsó G, Gábor M, Schönlau F, Rohdewald P. Pycnogenol® accelerates wound healing and reduces scar formation. Phyther Res. 2004;18:579-81. https://doi.org/10.1002/ptr.1477
4. D'Andrea G. Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications? Fitoterapia. 2010;81:724-36. https://doi.org/10.1016/j.fitote.2010.06.011
5. Becİt M, Aydin S, Başaran N. Pycnogenol in Human Health: An Overview. FABAD J Pharm Sci. 2017;42:125-38.
6. Dogan E, Yanmaz L, Gedikli S, Ersoz U, Okumus Z. The Effect of Pycnogenol on Wound Healing in Diabetic Rats. Osmoty Wound Manag. 2017;63(4):41-7.
7. Li X, Fan R, Tong A, et al. In situ gel-forming AP-57 peptide delivery system for cutaneous wound healing. Int J Pharm. 2015;495:560-71. https://doi.org/10.1016/j.ijpharm.2015.09.005
8. Okur NÜ, Yoltaş A, Yozgatli V. Development and Characterization of Voriconazole Loaded In Situ Gel Formulations for Ophthalmic Application. Turk J Pharm Sci. 2016;13:311-7.
9. Kranthi Kumar K, Swathi M, Srinivas L, Naseeb Basha S. Formulation and evaluation of floating in situ gelling system of losartan potassium. Der Pharm Lett. 2015;7:98-112.
10. Shashank Nayak N, Sogali BS, Thakur RS. Formulation and evaluation of pH triggered in situ ophthalmic gel of Moxifloxacin hydrochloride. Int J Pharm Pharm Sci. 2012;4:452-9.
11. Al Khateb K, Ozhmukhametova EK, Mussin MN, et al. In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery. Int J Pharm. 2016;502:70-9. https://doi.org/10.1016/j.ijpharm.2016.02.027
12. Qian Y, Wang F, Li R, Zhang Q, Xu Q. Preparation and evaluation of in situ gelling ophthalmic drug delivery system for methazolamide. Drug Dev Ind Pharm. 2010;36:1340-7. https://doi.org/10.3109/03639041003801893
13. Üstündağ-Okur N, Ege MA, Karasulu HY. Preparation and characterization of naproxen loaded microemulsion formulations for dermal application. Int J Pharm. 2014;4:33-42.
14. Üstündağ Okur N, Çağlar EŞ, Arpa MD, Karasulu HY. Preparation and evaluation of novel microemulsion-based hydrogels for dermal delivery of benzocaine. Pharm Dev Technol. 2017;22:500-10. https://doi.org/10.3109/10837450.2015.1131716
15. Okur ME, Ayla Ş, Çiçek Polat D, Günal MY, Yoltaş A, Biçeroğlu

Ö. Novel insight into wound healing properties of methanol extract of Capparis ovata Desf. var. palaestina Zohary fruits. J Pharm Pharmacol. 2018;70:1401-13. https://doi.org/10.1111/jphp.12977
16. Okur ME, Polat DC, Ozbek H, Yilmaz S, Yoltas A, Arslan R. Evaluation of the antidiabetic property of capparis ovata desf. Var. Paleastina zoh. Extracts using in vivo and in vitro approaches. Endocrine, Metab Immune Disord - Drug Targets. 2018;18:489-501. https://doi.org/10.2174/1871530318666180328110524
17. Galeano M, Altavilla D, Bitto A, et al. Recombinant human erythropoietin improves angiogenesis and wound healing in experimental burn wounds*. Crit Care Med. 2006;34:1139-46. https://doi.org/10.1097/01.CCM.0000206468.18653.EC
18. Ayla Ş, Günal MY, Sayın-Şakul AA, et al. Effects of Prunus spinosa L . fruits on experimental wound healing. Medeni Med J. 2017;32:152-8. https://doi.org/10.5222/MMJ.2017.152
19. Üstündağ Okur N, Filippousi M, Okur ME, et al. A novel approach for skin infections: Controlled release topical mats of poly(lactic acid)/poly(ethylene succinate) blends containing Voriconazole. J Drug Deliv Sci Technol. 2018;46:74-86. https://doi.org/10.1016/j.jddst.2018.05.005
20. Cetin EO, Yesil-Celiktas O, Cavusoglu T, Demirel-Sezer E, Akdemir O, Uyanikgil Y. Incision wound healing activity of pine bark extract containing topical formulations: A study with histopathological and biochemical analyses in albino rats. Pharmazie. 2013;68:75-80.
21. Tümen İ, Akkol EK, Taştan H, Süntar I, Kurtca M. Research on the antioxidant, wound healing, and anti-inflammatory activities and the phytochemical composition of maritime pine (Pinus pinaster Ait). J Ethnopharmacol. 2018;211:235-46. https://doi.org/10.1016/j.jep.2017.09.009
22. Ko J-W, Lee I-C, Park S-H, et al. Protective effects of pine bark extract against cisplatin-induced hepatotoxicity and oxidative stress in rats. Lab Anim Res. 2014;30:174-80. https://doi.org/10.5625/lar.2014.30.4.174
23. Li X, Fan R, Tong A, et al. In situ gel-forming AP-57 peptide delivery system for cutaneous wound healing. Int J Pharm. 2015;495:560-71. https://doi.org/10.1016/j.ijpharm.2015.09.005
24. Miguel SP, Ribeiro MP, Brancal H, Coutinho P, Correia IJ. Thermoresponsive chitosan-agarose hydrogel for skin regeneration. Carbohydr Polym. 2014;111:366-73. https://doi.org/10.1016/j.carbpol.2014.04.093
25. Hubbell JA. Hydrogel systems for barriers and local drug delivery in the control of wound healing. J Control Release. 1996;39:305-13. https://doi.org/10.1016/0168-3659(95)00162-X
26. Balakrishnan B, Mohanty M, Umashankar PR, Jayakrishnan A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials. 2005;26:6335-42. https://doi.org/10.1016/j.biomaterials.2005.04.012
27. Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. Acta Biomater. 2013;9:7081-92. https://doi.org/10.1016/j.actbio.2013.03.005
28. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm Res. 2006;23:2709-28. https://doi.org/10.1007/s11095-006-9104-4
29. Singh-Joy SD, McLain VC. Safety Assessment of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, Poloxamer 105 Benzoate, and Poloxamer 182 Dibenzoate as Use. Int J Toxicol. 2008;27:93-128. https://doi.org/10.1080/10915810802244595
30. dos Santos ACM, Akkari ACS, Ferreira IRS, et al. Poloxamer-based binary hydrogels for delivering tramadol hydrochloride: Sol-gel transition studies, dissolution-release kinetics, in vitro toxicity, and pharmacological evaluation. Int J Nanomedicine. 2015;10:2391-2401.
31. Garala K, Joshi P, Patel J, Ramkishan A, Shah M. Formulation and evaluation of periodontal in situ gel. Int J Pharm Investig. 2013;3:29. https://doi.org/10.4103/2230-973X.108961
32. Okur NÜ, Yozgatli V, Okur ME, Yoltaş A, Siafaka PI. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive in situ gels as ophthalmic drug carriers. J Drug Deliv Sci Technol. 2019;49:323-33. https://doi.org/10.1016/j.jddst.2018.12.005
33. Park JY, Kwak JH, Kang KS, et al. Wound healing effects of deoxyshikonin isolated from Jawoongo: In vitro and in vivo studies. J Ethnopharmacol. 2017;199:128-37. https://doi.org/10.1016/j.jep.2016.10.031