

Short Communication

## Polysaccharides isolated from *Phellinus gilvus* enhances dermal wound healing in streptozotocin-induced diabetic rats

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**Dermal wound healing is a complex process that involved inflammation leading to re-epithelialization, granulation tissue, and tissue remodeling. Previous studies from our laboratory have shown that polysaccharides isolated from fungus, *Phellinus gilvus* (PG) have various anti-inflammatory activities. In present study, we have assessed the effect of polysaccharides from PG on the dermal wound healing of polysaccharides from PG in streptozotocin-induced diabetic rat model. Six of 6-mm circular wounds were created with biopsy punch on the 4th day after induction of diabetes. After 24 hours, each test substance was applied to the wound twice a day for next 5 days. Circular wounds treated with PG showed significantly reduced wound contraction and complete re-epithelialization, as compared to wounds of non-treated ( $p < 0.05$ ). These results show that polysaccharides isolated from PG enhanced wound repair in diabetic impaired healing, and could be developed as a wound healing agent in such clinical settings.**

**Key words:** diabetes, *Phellinus gilvus*, polysaccharides, rat, wound healing

Tissue repair and wound healing are complex processes involving inflammation, re-epithelialization, neovascularization, fibroplasia, and wound contraction [8]. In the normal host, wound healing is usually uncomplicated and proceeds at a rapid rate. In contrast, most healing failures are associated with some form of host impairment, including diabetes, infection, immunosuppression, obesity, or malnutrition [14]. Among them, diabetes probably represents the prototype of impaired healing model since contribution of impairment such as peripheral vascular disease and lowered immunity against infections. Therefore, we employed streptozotocin-induced diabetic rat model for our study.

Several natural products have been investigated in the

promotion of wound healing of normal or abnormal host. The extract of *Centella asiatica* (Madecassol; Dong Kook Pharm, Korea) used in this study is a well-known medical ointment in enhancing of dermal wound healing [15,16]. Recently, polysaccharides isolated from *Phellinus* spp. have received special attention due to their potent pharmacological activities such as anti-tumor [4,9] and anti-inflammatory [11]. We have previously demonstrated that polysaccharides isolated from the *Phellinus gilvus* (PG) have various biological activities related to inflammation, including inhibition of pulmonary inflammation [10], prevention of intraperitoneal adhesion under infectious circumstances [1,3,5], and promotion of dermal wound healing in normal host [2]. In this work, we investigated whether polysaccharides isolated from PG could enhance dermal wound healing in streptozotocin-induced diabetic rats.

Ten male Sprague-Dawley rats (243–285 g) were purchased from Charles River Laboratory (BioGenomics, Korea) and used to carry out experiments after an acclimatization period of 7 days in controlled room. The animals were fed with commercial rat diet (Orient, Korea). Food and water were provided *ad libitum*. All animals were used in compliance with the *Guidelines for Animal Care and Use* [13]. Diabetes was induced by a single intraperitoneal injection of streptozotocin (70 mg/kg body weight, Sigma, USA) in 0.1 M citrate buffer, pH 4.0. Fasting blood glucose levels were checked with glucose oxidase reagent strips before and 3 days after streptozotocin injection, and rats with consistent blood glucose levels higher than 200 mg/dl were used for this study.

The fruiting body of PG was kindly provided by Gyeongbuk Agricultural Technology Administration (Daegu, Korea) and grown rapidly for 3 months in artificial oak sawdust cultures. It was homogenized, extracted by optimal water extraction conditions, distilled water (1 : 25) at 100°C for 10 hours, and concentrated at 80°C in a rotary evaporator. The recovery procedure of the polysaccharides solution from the fruiting body of PG followed an established method in our previous studies [1–5]. The concentration of 0.025, 0.25, and 2.5% polysaccharides solutions was determined by total sugar according the

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anthrone method [6] using glucose as the standard material. These solutions formulated as a suspension in a small of aqueous gel (Biosonic; Amitie, Korea) and Madecassol.

Wounds were created on the 4th day after induction of diabetes. Animals were anesthetized using Rompun (10 mg/kg) and Ketamine (200 mg/kg). Hair on the dorsal side of the rats was shaved and the skin was cleaned with 70% ethanol. A 6-mm skin biopsy punch was used to create six of circular wounds under aseptic conditions. The wound were made 1.25 cm to the right and left sides of the midline and separated from cranial or caudal by 2.5 cm. At 24 hours postoperatively, each test substance were applied to the circular wound, respectively. Application of test substances was continued twice a day for the next 5 days. Test substances would be applied at a different wound location in each animal. The substances were as follow: 0.025, 0.25, and 2.5% polysaccharides isolated from PG (PG0.025, PG0.25, and PG2.5 group), Madecassol (MC group), aqueous gel (AG group) and no treatment (control group). No dressing was placed on any of the wounds. Animals were sacrificed 6 days postoperatively (5 days after initial treatment by test substance). Wound radius was determined by measuring craniocaudal and lateromedial distance. An average of those two measurements was obtained and used to predict wound diameter.

Wound tissue was collected at the time of euthanasia and fixed in 10% buffered formalin. A 5  $\mu$ m thick sections were cut and stained with hematoxylin and eosin. All subsequent analyses were performed by observers blinded to treatment. Histological sections were used to observe the reepithelialization, congestion, edema, infiltration of polymorphonuclear leukocytes and monocytes, necrosis, fibroblastic proliferation, collagen formation and angiogenesis. The degree of reepithelialization was estimated as % of incision width reepithelialized in each wound tissue. Analysis of differences between treated groups and untreated groups was performed using analysis of variance followed by multiple comparisons and Fisher's LSD test using the SAS statistical package (release 8.1; SAS, USA). Differences at  $p < 0.05$  were considered statistically significant.

Contraction of wound and re-epithelialization of tissue on any of the treatment had no quantitative effect according to location of wounds. Gross observation of the punch wounds at the time of euthanasia showed that all PG treated wounds were healthier, in that they were not oozing and appeared well contracted unlike many control wounds in diabetic rats. The most dramatic result of wound diameter were seen in PG2.5 group compared to control group ( $p < 0.05$ ). Predicted wound diameter of control and each experimental group by measuring craniocaudal and lateromedial distance are presented in Table 1.

The microscopic examination of wound the dermis showed proliferation of fibroblasts, hemorrhage, angiogenesis and infiltration of polymorphonuclear leukocytes and

**Table 1.** Wound diameter predicted by measuring craniocaudal and lateromedial distance at the time of euthanasia in diabetic rats (n = 10) (mean  $\pm$  SD)

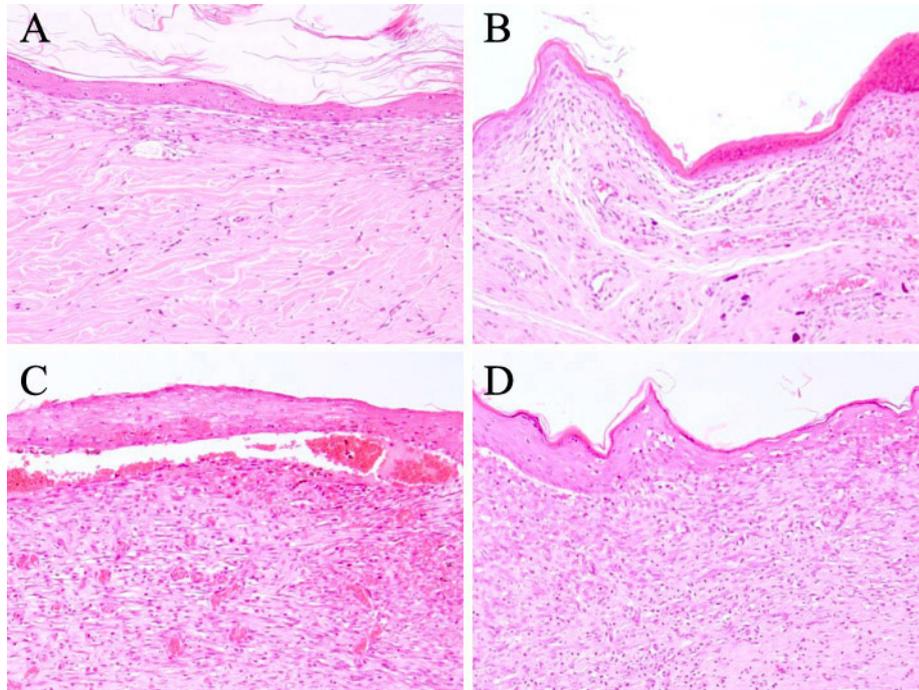
Groups	Predicted wound diameter (mm)
Control	4.24 $\pm$ 0.57
MC	3.65 $\pm$ 1.31
PG0.025	3.52 $\pm$ 0.47 <sup>b</sup>
PG0.25	3.11 $\pm$ 0.84 <sup>b</sup>
PG2.5	2.61 $\pm$ 0.39 <sup>a</sup>
AG	3.74 $\pm$ 1.23

<sup>a</sup> $p < 0.05$  compared with all groups was significant. <sup>b</sup> $p < 0.05$  versus control group. Control group (no treatment), MC group (Madecassol), PG0.025, PG0.25, and PG2.5 group (0.025, 0.25, and 2.5% polysaccharides gel isolated from PG) and AG group (aqueous gel).

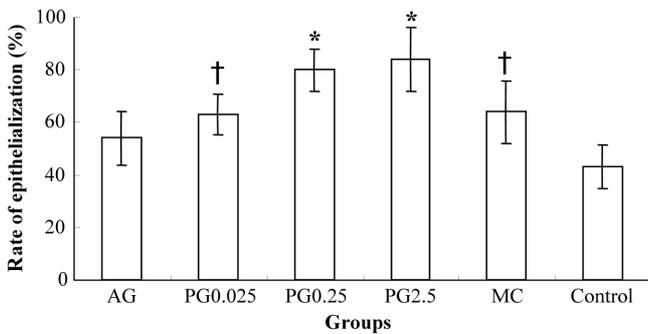
monocytes, degree of which parameters did not show significant difference between each groups (Fig. 1). PG treated wounds of diabetic rats showed an increase in the rate of re-epithelialization. Complete re-epithelialization was noted in PG2.5 group. In MC group, about 60% of incision width was re-epithelialized. In control group, only 40% of the wound was re-epithelialized. Crust of necrotic debris with inflammatory cells covered denuded epidermis and the dermis below showed marked edema and congestion (Fig. 1). The rate of re-epithelialization (%) of the control group was 43  $\pm$  8.3. The rate of PG2.5 (84  $\pm$  11.9) and PG0.25 (80  $\pm$  7.9) group was higher than that in control and AG (60  $\pm$  7.9) group ( $p < 0.05$ ) (Fig. 2). PG0.025 (63  $\pm$  7.6) and MC group (64  $\pm$  11.9) was slightly higher than control group. There was no statistically significant difference in the rate of re-epithelialization of the AG and control group.

The results of the present study showed that all polysaccharides isolated from PG enhance significantly wound healing in streptozotocin-induced diabetic rats. Previous studies from our laboratory reported that the polysaccharides have effect of dermal wound healing in the normal host [2]. Thus, we conclude that the polysaccharides may enhance wound healing both not only normal but also diabetic host. PG used in our study is fungi belonging to the *Hymenochaetaceae basidiomycetes* [7] and it is commonly referred to as Sangwhang in Korea. The effects of PG related to various pharmacological activities have demonstrated continually. In addition, it has advantages over the other *Phellinus* mushrooms in that it has a very short growth period (3 months) and need it cheaper to produce. Therefore, we can predict that use of PG will be increased gradually as a functional food and medical supplements in the future.

We have previously demonstrated that polysaccharides isolated from PG inhibit pulmonary inflammation [10] and intraperitoneal adhesion related to intraperitoneal inflammation [1,3,5]. These effects of PG related to anti-inflammatory activities might be beneficial in the treatment of dermal



**Fig. 1.** Histopathologic appearances of wounds at the time of euthanasia in streptozotocin-induced diabetic and normal rats. The wounds showed dermal proliferation of fibroblasts and mononuclear inflammatory cells with angiogenesis. Re-epithelialization was only 40% in control group (A) and about 60% in MC (Madecassol, B) and PG0.025 (C). Crust covered denuded epidermis and the dermis below showed marked edema. PG2.5 group (D) showed complete re-epithelialization with increased number of epithelial cell layer, thick granular layer, hyperkeratosis and parakeratosis. H&E stain,  $\times 100$ .



**Fig. 2.** Rate of re-epithelialization of control and experimental wounds (n = 10). \* $p < 0.05$  versus all groups and †  $p < 0.05$  versus AG and control group.

wound healing. In the studies, we showed that PG is a potent macrophage stimulator that enhances macrophage cytotoxicity and phagocytic capacity. Macrophages is known to play a key role in wound repair. Leibovich and Ross [12] reported that healing was delayed when wound macrophages were depleted. Therefore we may conclude that dermal wound healing may be promoted by modulating the macrophage activity of polysaccharides isolated from PG.

In summary, polysaccharides isolated from PG enhanced wound repair in diabetic impaired healing, and could be

developed as a wound healing agent in such clinical settings. Additional studies regarding its mechanism of action will help further reveal of its uses and limitations.

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