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Wound Healing Effectiveness Test of Dermal Patch Formulated with Green Synthesized Silver Nanoparticles from *Plantago major* L. Extract

Johan Sukweenadhi^{1*}, Stefan Pratama Chandra¹, Finna Setiawan², Christina Avanti², Kartini Kartini², Arief Koeswanto³ and Deok-Chun Yang⁴

¹Department of Plant Biology, Faculty of Biotechnology, University of Surabaya, Jalan Raya Kalirungkut, Kalirungkut, Surabaya, East Java 60293, Indonesia; ²Department of Pharmaceutics, Faculty of Pharmacy, University of Surabaya, Jalan Raya Kalirungkut, Kalirungkut, Surabaya, East Java 60293, Indonesia; ³PT Kurnia Cahaya Teknik, Jakarta 14410, Indonesia; ⁴Graduate School of Biotechnology, College of Life Science, Kyung Hee University, Yongin-si, Gyeonggi-do 17104, Republic of Korea.

Abstract | Skin is the largest organ in the body and has a very important role. A wound is an injury in which the skin is torn, cut, punctured, or traumatized. Wounds in diabetic patients are difficult to heal. Green synthesis is one of many methods that can be used to synthesize silver nanoparticles. Based on a statistical analysis of the average wound area, epidermal reconstruction, and TIME-H scores, significant differences began to occur from day 12 to day 14. The average wound area, epidermal reconstruction, and TIME-H scores in the diabetic mice group that were given a dermal patch formulated with silver nanoparticles synthesized using *Plantago major* L. extract were not significantly different from the normal group and the ACA and ACA Ag groups. Based on these results, it can be concluded that the dermal patch formulated with silver nanoparticles synthesized using *Plantago major* L. extract helps wound healing in diabetic mice and is not significantly different from wound dressings and silver dressings on the market (p-value < 0.05). In addition, the best concentration that can be formulated into the dermal patch is 0.005% (p-value <0.05).

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*Correspondence | Johan Sukweenadhi, Department of Plant Biology, Faculty of Biotechnology, University of Surabaya, Jalan Raya Kalirungkut, Kalirungkut, Surabaya, East Java 60293, Indonesia; Email: sukwee@staff.ubaya.ac.id

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Introduction

Skin is the largest organ in the body and has a very important role in sensory function, homeostasis, body temperature regulation, protection against pathogens, toxins, and trauma. A wound is an injury in which the skin is torn, cut, punctured, or traumatized by a blunt object that can cause discolorations.

Generally, wounds can be classified into 2, namely acute wounds and chronic wounds (Paladini and Pollini, 2019). The wound healing process is divided into 3 stages: Inflammation, proliferation, and remodeling/maturation. Every wound must go through these three stages to heal completely, with the inflammation stage being the key stage and have a vital role in the local immune response (Wang *et al.*, 2022).





The wound healing process is very efficient, but, the healing process can be disrupted in certain circumstances. In America, there are ± 6 million people with diabetes who have problems with wound healing. Wounds in DM patients generally do not dry properly due to excessive exudate production. Wounds that are difficult to heal become a strategic place for microorganisms to increase and will be one of the many factors that contribute to causing a chronic wound. One way to overcome this problem is to acquire a topical preparation with the following criteria can protect wounds from microorganisms, remove excess exudate produced by wounds and toxins, keep the wound moist, permit gas exchange, clean easily, and inexpensive (Alexiadou and Doupis, 2012; Kartini et al., 2020). Other study found that a hydrocolloid film containing Vicenin-2, a natural compound with antioxidant and anti-inflammatory properties, can enhance diabetic wound healing in a dose-dependent manner (Tan et al., 2019). Sodium alginate, a natural polysaccharide with gel-forming properties, can also be used as a wound dressing to promote wound healing (Renuka et al., 2023). However, it's important to note that the effectiveness of topical preparations can vary depending on the type and severity of the wound, and it's always best to consult with a healthcare professional before using any new treatment.

In current medicine, the best and most commonly used way to deliver a drug is through the skin using patches. Patches contain ultra-concentrate in a very small form where this patch will stick to the skin. In addition, dermal patches have been proven to be the fastest, easiest, safest, and cheapest in wound healing. One of the most commonly used dermal patch formulations is film. Natural polymeric films combining zen and pectin show potential as drug delivery systems for treating wounds and inhibiting pathogen growth (Fiorentini et al., 2021). This formulation aims to increase thpatch's water resistance, improve cosmetic properties, and reduce patient complaints. Previous study reported the optimized formulation of Centella extracts loaded with aloe vera gel, 20% glycerine and 15% sodium alginate in transdermal patched can produces good films with controlled physical properties for wound healing (Puttarak et al., 2015). Drugs can be in the form of a spray, gel, or emulsion. The film has several components: active ingredients, polymers, solvents, and plasticizers (Pünnel and Lunter, 2021).

Green synthesis involves microorganisms, plants, viruses, and animal cells to synthesize nanoparticles. The synthesis of nanoparticles using the green synthesis method has several advantages, such as being eco-friendly, time affordable, and cost-effective (Pirtarighat *et al.*, 2019). The utilization of plants in the biosynthesis of nanoparticles involves secondary metabolites contained in plants as reducing agents. Secondary metabolites can act as reducers, stabilizers, or both in the formation of nanoparticles (Aritonang *et al.*, 2019). Recent studies have shown that plantmediated synthesis of silver nanoparticles using different plant extracts can be an effective way to produce nanoparticles with antimicrobial properties (Vanlalveni *et al.*, 2021).

Silver nanoparticles are interesting metals to study in health and medicine. Silver has very strong antibacterial properties because it can damage cell walls inhibit the development of bacterial cells, and interfere with metabolism in bacterial cells. After all, silver interacts with macromolecules in cells (Aritonang *et al.*, 2019). The use of silver as an antibiotic agent has the advantage that it cannot cause resistance to microorganisms, so it can be used in the long term. Because silver has a broad spectrum of antimicrobial activity, it can be used to overcome the problem of antibiotic resistance in microorganisms. In recent years silver has been widely applied in various fields, namely wound dressings, artificial implantation, and careers in tumor treatment (Paladini and Pollini, 2019).

Plantago major L. has been widely used to treat many ailments, such as constipation, coughs, and wounds. Plantago major L. contains various secondary metabolites, such as flavonoids, polysaccharides, terpenoids, lipids, iridoid glycosides, and caffeine acid derivatives (Najafian et al., 2018). Due to the large number of secondary metabolites possessed by Plantago major L., this plant can be used to synthesize nanoparticles, reduce metal salts, and act as a capping and stabilizing agent. According to research by Mahmood and Phipps (2006), the aqueous extract of Plantago major leaves has wound healing activity when applied to rats (Mahmood and Phipps, 2006).

Previous reported work have synthesized silver nanoparticles using *Plantago major* L. leaf extract (Kartini *et al.*, 2020). Recent scientific studies have shown that silver nanoparticles synthesized using *Plantago major* L. leaf extract can be used as an effective





wound healing agent. Another study found the optimal concentration of silver nanoparticles required to promote wound healing (Vijayaram *et al.*, 2023). The purpose of this study was to determine the activity of a dermal patch formulated with silver nanoparticles synthesized using *Plantago major* L. extract in helping to heal wounds in diabetic mice and to determine the best concentration of silver nanoparticles to help heal wounds in diabetic mice model.

Materials and Methods

Induction of diabetic mice

42 Male mice (Swiss Webster) were acclimatized for ± 2 weeks. Feeding and drinking are done ad libitum. This injection of streptozotocin (STZ) refers to the journal Furman (2021) with a slight modification. STZ (125 mg/ Kg body weight) dissolved in 0.9% NaCl sterile. STZ injection was done once on day-0 intraperitoneally. During the injection process, the STZ solution was kept in an ice bath to prevent the breakdown of STZ. Mice will be tested for the clinical parameters: Fasting Blood Glucose (FBG). FBG will be tested before STZ injection and day-7 after STZ injection (Tang et al., 2014).

Effectiveness test of dermal patch formulated with silver nanoparticles

Dermal patch formulated silver nanoparticle effectiveness test using the method described in Nayak and Gupta (2017) with slight modifications. The first step was to remove fur in mice using fur removal cream, allowing it to stand for 1 night. After fur removal, mice were anesthetized using ketamine hydrochloride (100 mg/kg Body Weight) intraperitoneally. The skin was injured with a wound diameter of ±0.5 cm. Mice were divided into 7 groups, namely a group of normal mice without treatment (normal), a group of diabetic mice without treatment (induction), a group of diabetic mice using algelle calcium sodium alginate (induction ACA), a group of diabetic mice using algelle Ag (induction ACA Ag), a group of diabetic mice using a placebo (induction

DPP), a group of diabetic mice using a dermal patch formulated with 0.005% silver nanoparticles produced by a green synthesis of *Plantago major* L. extract (induction DP0005), and a group of diabetic mice using a dermal patch formulated with silver nanoparticles. 0.01% yield of green synthesis of *Plantago major* L. extract (induction DP001). The treatment was given every day and applied according to the wound's area on the mice's back. Observations were conducted for 14 days. Wound images were taken on the 4th, 8th, 12th, and 14th days.

Fasting blood glucose test after effectiveness test

Fasting Blood Glucose (FBG) test was carried out after testing the effectiveness of the silver nanoparticle dermal patch following the method contained in the FBG protocol. Mice were fasted for 12 hours (overnight) by replacing the old sawdust with new sawdust and emptymice'se mice'se's feeding area. After that, fasting blood sugar levels were measured using a glucometer and the strip. Mice were declared to have diabetes when fasting blood glucose levels reached over 150 mg/dL. Diabetic mice will be used for the dermal patch formulated silver nanoparticle effectiveness test.

Data analysis

Analysis of the effectiveness of dermal patches formulated with silver nanoparticles in wound healing was carried out by comparing the data on wound area, epidermal reconstruction, and the wound score (Table 1). The area and epidermal reconstruction were calculated using AlphaEase software, while the wound score was analyzed using the TIME-H (Tissue, Inflammation/infection, Moisture, and epithelization) method. Data analysis used IBM SPSS statistics 25 software. Wound area, epidermal reconstruction, and TIME-H scores were analyzed parametrically if data met the parametric test requirements. If it does not meet the requirements, a non-parametric test will be carried out. The test results are significantly different if the p-value <0.05. Wound values were performed using the TIME-H method with the following scoring system (Lim et al., 2015).

Table 1: Scoring system for wound assessment.

8 2 3			
Wound score	0	1	2
Tissue necrosis (%)	0	<50	>50
Infection	Contamination	Colonization	Infection
Moisture	No exudates	Exudates exists	Smelly exudates
Epidermal reconstruction	>90	90-30	<30

The tested dermal patches could be declared effective in helping to heal wounds if the average wound area, epidermal reconstruction, and TIME-H scores were not significantly different from the normal, ACA, and ACA Ag groups or significantly different from the induction group.





Result and Discussion

Induction of diabetic mice

Induction of Diabetes Mellitus (DM) in male Swiss Webster mice was carried out for 7 days. On day 0, mice were injected intraperitoneally using STZ (125 mg/kg BW). The average fasting blood sugar levels of the normal and diabetic mice group can be seen in Figure 1. On day 0, the normal group's average fasting blood glucose level (68 mg/dL) was not different from the average fasting blood glucose level of the diabetic group (76 mg/dL). The difference in an average fasting blood glucose level happened on day 7, when was the normal group, 76 mg/dL, and the diabetic group 363 mg/dL. Based on the statistical analysis results, the average fasting blood glucose level of the normal group and diabetes groups were not significantly different on day 0. A significant difference occurred on day 7: The average fasting blood glucose level of the normal mice group was lower than the fasting blood sugar level of the diabetic mice group.

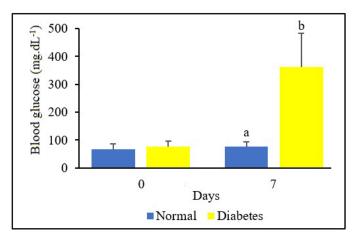


Figure 1: Bar chart to show the average fasting blood glucose of mice for each treatment on day-0 and day-7 after STZ injection. The difference in letters indicates a significant difference between the treatment groups ($\alpha = 5\%$).

High fasting blood glucose (363±123 mg/dL) in the diabetes group was caused by STZ injection. STZ selectively destroys pancreatic cells so that insulin cannot be produced by the pancreas (Goud *et al.*, 2015). In addition, mice in the normal group were more active in moving than mice in the diabetes group even though they had the same fasting duration, namely 12 hours. Decreased activity is also caused by the absence/lack of the hormone insulin. The insulin hormone functions to induce glucose into muscle cells so that when there is no insulin, muscle cells will lack energy, which impacts the activity of mice (Perry *et al.*, 2014). Based on the results of the GDP test on

day 7 and observations of the behavior of mice, it can be concluded that mice in the diabetes group have diabetes (Furman, 2021; Sharma et al., 2010).

Effectiveness test of dermal patch formulated with silver nanoparticles

The dermal patches used in this study were sourced from the Faculty of Pharmacy, University of Surabaya. They created a formulation in the form of hydrogel film. Hydrogel film has the advantage of a cold sensation when applied to the wound because it is water-based, allows gas exchange, absorbs excess exudate, and does not stick firmly to the wound. In addition, hydrogel films have been used quite often to deliver drugs in skin treatment (Brumberg et al., 2021; Zagórska-Dziok and Sobczak, 2020). This film is based on a hydrogel with a composition of silver nanoparticles, Hydroxypropyl Methyl Cellulose (HPMC), carbomer 974p, distilled water, and glycerin. HPMC is a hydrophilic polymer and is widely used in the pharmaceutical industry. HPMC can be used as a binder, gelling agent, thickening agent, hydrophilic matrix, and film former. This HPMC can produce transparent, flexible and strong films (Ode et al., 2021; Panraksa et al., 2020). The use of carbomer 974p has the same purpose as HPMC, namely as a gelling agent in manufacturing hydrogel films. In addition, it is necessary to add glycerol as a plasticizer to increase the elasticity of the hydrogel film (Panraksa et al., 2020).

Green synthesis is one of many methods that can be used to produce silver nanoparticles. Green synthesis involves secondary metabolites to reduce metal ions to their metallic forms. Green synthesis has many advantages over physical and chemical methods, namely eco-friendly, affordable time, and costeffectiveness, and has a uniform silver nanoparticle size (Pirtarighat et al., 2019). This green synthesis method can overcome problems regarding production speed, yield, environmental safety, and uniformity of silver nanoparticle size (Zhang et al., 2016). The size of the silver nanoparticles produced from Plantago major L. extract was 10.3±3.98 nm, round in shape and had a Polydispersity Index (PDI) value of 0.15. The lower the PDI value, the more uniform the nanoparticle size will be, and vice versa (Sukweenadhi et al., 2021). The size of silver nanoparticles produced by *Plantago major* L. is smaller when compared to the size of silver nanoparticles produced by other plants such as Phyllanthus niruri, Orthosiphon stamineus, and



Table 2: The appearance of wounds in each group on 0 to 14 days during the dermal patch effectiveness test.

Treatment	Day 0	Day 4	Day 8	Day 12	Day 14
Normal	Letter visits	The second secon	20 The State of th	20 Department of the second of	28 Names and a second s
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Based on statistical analysis, the average epidermal reconstruction for each group did not significantly differ from day 0 to day 8. Significant differences occurred on day 12 and day 14. The average epidermal reconstruction of the normal, induction ACA, induction ACA Ag, induction DPP and induction DP0005 groups were significantly different than the induction DP001 and induction groups. There was no significant difference between the normal, induction ACA, induction ACA Ag, induction DPP, and induction DP0005 groups and there was no significant difference between the induction and the induction DP001 group.



Curcuma longa (Kartini et al., 2020). The smaller size allows for an increase in the ratio of surface area to volume. This causes more silver nanoparticles, which can later interact directly with bacteria and pathogens found in wounds (Aritonang et al., 2019). In addition, some studies show that the smaller the size of a particle, the aaaaanti-bacterial activity of the particle will increase (Ferdous and Nemmar, 2020).

The effectiveness test of dermal patch formulated with silver nanoparticles was carried out by comparing the average the wound area, epidermal reconstruction, and TIME-H scores on normal group wounds and on wounds that had been treated using wound dressings and silver dressings available on the market. Observations were conducted for 14 days with 5 times of data collection, that is, 0, 4th, 8th, 12th, and 14th days (Table 2).

Visually, the wound area in the normal group, ACA, ACA Ag, DPP, and DP0005, healed on day 14. In the normal group, ACA, ACA Ag, DPP, and DP0005, the wounds began to shrink on day 4, but the shrinkage in the normal group was faster than the ACA, ACA Ag, DPP, and DP0005 groups. In the induction group, the wound area did not shrink from day 0 to day 14. Even though the wound area was getting bigger. In the DP001 group, the wound area decreased on day 12 and day 14, but the wound area was still quite large compared to the normal group, ACA, ACA Ag, DPP, and DP0005.

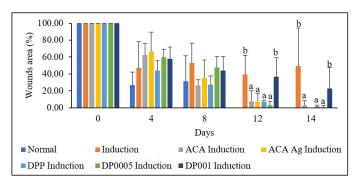


Figure 2: Bar chart to show average of wound area on each group for day 0, day 4, day 8, day 12, and day 14. The difference in letters indicates a significant difference between the treatment groups ($\alpha = 5\%$).

Figure 2 shows the average wound area of mice for each treatment on day 0, 4, 8, 12, and 14. Significant differences in the average wound area began to occur on day 12. Meanwhile, the normal group had the fastest wound shrinkage rate compared to the other groups because on day 12, the wound area had reached 0%.

This is following the theory that the wound healing process under normal circumstances is efficient and can occur in parallel between one stage and another (Saghazadeh et al., 2019). The induction group had the largest average wound area, 39.49±22.61%, which is in accordance with the theory that DM inhibits the wound healing process by preventing the wound from entering the proliferative stage and remaining in the inflammatory stage. Many inflammatory compounds such as Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha and (TNF- α) can be found in wound areas (Younis et al., 2022). Based on research conducted by Younis et al. (2022), wounds in DM mice that are not treated properly will still exist until the 21st day. The ACA and ACA Ag groups had a fairly small average wound area, namely 7.28 ±12.62% and 6.82±10.24%. This could be because DM mice used wound dressings and silver dressings which have been commonly used to help heal wounds of DM patients. The use of wound dressings and silver dressings in the ACA and ACA Ag groups is able to prevent contamination of microorganisms, remove excess exudate produced by wounds, keep wounds moist, impermeable to microorganisms, and do not hinder gas exchange.

The DPP and DP0005 groups had a relatively small mean wound area, 6.89±1.76% and 2.77±4.80%, respectively. Dermal patches can help the wound healing process by preventing infection, removing excess exudate from the wound, and keeping the wound moist. The addition of silver to ACA Ag and DP0005 can help heal wounds better. This is because these two groups have a smaller average wound area than ACA and DPP. Silver can kill microorganisms found in wounds through several mechanisms, including interfering with cell wall permeability, producing Reactive Oxygen Species (ROS), penetrating cell membranes, and binding to DNA and proteins (Sukweenadhi *et al.*, 2021).

Moreover, the interesting thing that can be found in the difference in the concentration of silver in the silver dressing and the concentration of silver nanoparticles in the dermal patch. The concentration of silver used in silver dressings (1%) was higher than that of silver nanoparticles used in dermal patches (0.005%), but the results were not significantly different. Although the mechanism of silver nanoparticles to kill bacteria is still unclear, These data show that silver nanoparticles are better at killing microorganisms because they are





only required in small concentrations. This can happen because the silver in the form of nanoparticles can increase the surface area to volume ratio so that more silver nanoparticles interact with bacteria and wounds (Aritonang *et al.*, 2019). The DP001 group had a large average wound area of $36.67 \pm 22.48\%$. This is presumably due to the toxic effect caused by using silver nanoparticles with a fairly large concentration, which is 0.001%. Silver nanoparticles can inhibit the work of fibroblasts, where fibroblast cells are one of the cells that are important in the proliferation stage (Khansa *et al.*, 2019; Saghazadeh *et al.*, 2019).

The DP001 group's decrease in the average wound area could be attributed to the dermal patch's composition, which was designed to prevent infection, remove excess exudate from the wound, and maintain wound moisture. However, this result differs from Younis et al. (2022) findings that increasing the concentration of silver nanoparticles accelerates wound closure. This is because a higher concentration of silver nanoparticles (10 g/kg to 30 g/kg) increases collagen production (Younis et al., 2022). Differences in experimental animals, namely mice and rats, may have caused the difference in results. Experimental animals' tolerance to silver nanoparticles varies (Younis et al., 2022). On day 14, the normal group, ACA induction, ACA Ag induction, DPP induction, and DP0005 had significantly smaller average wound areas than the induction and DP001 induction groups.

The next parameter analyzed was the epidermal reconstruction. Epidermal reconstruction occurred from day 4 to day 14, but each treatment had a different speed of epidermal reconstruction. Based on statistical analysis, the average epidermal reconstruction for each treatment from day 0 to day 8 was not significantly different. Significant differences began to occur on day 12. In the normal group, the epidermis reconstruction was 100%, indicating that the wound healing stage of the normal group had entered the maturation stage. The maturation stage aims to achieve maximum strength of the formed tissue through reorganization, degradation, and re-synthesis of the extracellular matrix. When the wound has closed, there will be a change in the type of collagen in the wound, namely, type III collagen is replaced by a stronger type I collagen (Gonzalez et al., 2016). Collagen plays a key role in each phase of wound healing by attracting cells such as fibroblasts and keratinocytes to the wound, which encourages debridement, angiogenesis, and reepithelialization (Pallaske *et al.*, 2018).

The induction group had a small average epidermal reconstruction, which was 60.51±22.61%. This is presumably caused by the large number of inflammatory compounds in the injured area, which interfere with the epidermal reconstruction process in DM mice. The average epidermal reconstruction in the induction ACA and induction ACA Ag groups was quite large, namely 92.72±12.62% and 93.18±10.24%, respectively. This proves that the wound dressing and silver dressing used can help DM wounds pass the inflammatory stage and enter the proliferative stage so that epidermal reconstruction can occur. Epidermal reconstruction in the induction ACA Ag group was higher than in the induction ACA group, presumably due to the anti-bacterial and anti-fungal activity of silver, which can help the body to kill microorganisms in wounds, as previous reported studies (Hilton et al., 2004; Finley et al., 2013).

The induction DPP and induction DP0005 groups had a significant epidermal reconstruction, 93.11±1.76% and 97.23±23%, respectively. This suggests that the placebo has components that can help DM wounds undergo epidermal reconstruction. The addition of synthesized silver nanoparticles using Plantago major L. extract with a concentration of 0.005% on the dermal patch also accelerated the epidermal reconstruction process compared to the induction DPP group, although not significantly different. Silver nanoparticles have been shown to increase the production of compounds that play a role in the angiogenesis process, namely Hypoxia Inducible Factor-1 alpha (HIF-1α), Transforming Growth Factor-beta 1 (TGF-β1) and Vascular Endothelial Growth Factor (VEGF) (Liu et al., 2020). The induction DP001 group also had a small average epidermal reconstruction, which was 63.33±22.48% (Figure 3). This is thought to be caused by the toxic effect of silver nanoparticles on fibroblasts in the wound area, thereby inhibiting the wound healing process. Some studyconcludes that silver nanoparticles can induce a toxic response in different mammalian cell lines, resulting in decreased viability or the release of lactate dehydrogenase (Sambale et al., 2015). On day 14, the average wound area of the normal group, ACA induction, ACA Ag induction, DPP induction, and DP0005 remained significantly smaller than the induction and DP001 induction groups.

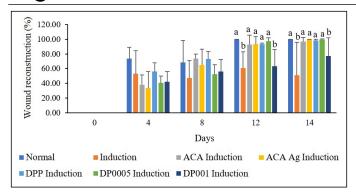


Figure 3: Bar chart to show average of epidermal reconstruction on each group for day 0, day 4, day 8, day 12, and day 14. The difference in letters indicates a significant difference between the treatment groups ($\alpha = 5\%$).

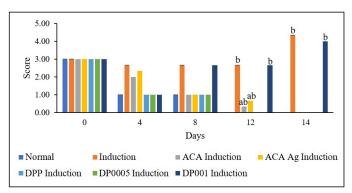


Figure 4: Bar chart to show average of TIME-H on each group for day 0, day 4, day 8, day 12, and day 14. The difference in letters indicates a significant difference between the treatment groups ($\alpha = 5\%$).

The last analyzed parameter was TIME-H. TIME-H is one method to determine the appropriate prognosis and treatment. TIME-H uses a healing score that will be given based on the condition of the wound, such as Tissue necrosis, Infection, Moisture, and Epithelialization. The lower score indicates that the wound is improving, and vice versa (Lim et al., 2015). The average TIME-H score for each treatment on day 0, 4, 8, 12, and 14 can be seen in Figure 4. Based on statistical analysis, the average score for each treatment on day 0, 4, and 8 decreased but did not significantly change. A significant difference occurred on day 12. The average score in the normal group has reached a value of 0. This indicates that the wounds in the normal group have healed perfectly. This further strengthens the theory that the healing process under normal conditions is efficient because the inflammatory stage can occur in parallel with the proliferative stage and the proliferative stage with the maturation stage (Saghazadeh et al., 2019).

Wounds in the normal group had reached the stage of maturation and can last long enough to create a strong epidermis as it was before the wound occurred. The scores of the induction ACA and induction ACA Ag groups were not significantly different from those of the induction and induction DP001 groups. This is because one mouse has epidermal reconstruction <90%, namely 78% and 81%, respectively. This is thought to be caused by environmental factors that slow down the healing process, namely stress. Stress can inhibit the production and disrupt the regulation of the Fibroblast Growth Factor (FGF), Endothelial Growth Factor (EGF), VEGF, and TGF-β so that the wound healing process takes longer (Guo and DiPietro, 2010). However, the scores in the induction ACA and induction ACA Ag groups were already very low, so it can be concluded that the wounds in both groups were getting better. The average score of the induction DPP and induction DP0005 groups has reached 0, which proves that each mouse has a very small wound area and there is no slough in the wound. The average score of the induction and induction DP001 groups was quite large, namely 2.67±2.89. This is because in the induction group and DP001, slough occurs. Slough is a thin, pale yellow layer on the wound. This slough can be wet or dry. Slough consists of leukocytes, dead cells, microorganisms, and exudate from the inflammatory reaction. Generally, this slough only occurs in wounds for ± 3 days but in the induction group and slough is still present on day 12 (Percival and Suleman, 2015). This proves that on day 12, there were still dead cells, microorganisms, and exudate in the wound, which was not found in the other groups. This slough is thought to be caused by excess exudates in the wound caused by an inflammatory reaction so that it becomes a place for bacteria to live (Kartini et al., 2021).

On day 14, the average TIME-H score for the normal, induction ACA, induction ACA Ag, induction DPP, and induction DP0005 had reached 0 while in the induction and induction DP001 groups was getting bigger (>4). A score of 0 indicates the condition of the wound has almost completely healed. However, the presence of exudate due to inflammatory and bacterial reactions is still a hypothesis. Further testing is needed to determine the content of exudate and the presence of bacteria in the wound. Analysis of the composition of the exudate content in the wound can be done by ELISA analysis. ELISA can analyze the presence of pro-inflammatory compounds such as IL- 1β , IL-6, and TNF- α . The presence of bacteria in the wound can be analyzed using microorganism analysis





techniques such as morphological and biochemical analysis.

FBG test after effectiveness test of silver nanoparticles dermal patch

After testing the effectiveness of the dermal patch formulated with synthetic silver nanoparticles using Plantago major L. extract, FBG was conducted to measure fasting blood glucose levels in the remaining mice. Fasting blood glucose levels were measured on the 19th day after the dermal patch effectiveness test. The average fasting blood glucose levels before and after the dermal patch test can be seen in Figure 5. Before the dermal patch effectiveness test, the normal group's fasting blood glucose levels were lower than the diabetic group's fasting blood glucose levels, which were 76 mg/dL and 363 mg/dL, respectively. On the 19th day after the dermal patch effectiveness test started, the normal group's average fasting blood glucose level was still lower than the average fasting blood glucose level of the diabetic group, which was 61 mg/dL and 275 mg/dL, respectively. Statistical analysis showed that the average fasting blood glucose levels of normal mice and diabetic mice were significantly different on day 0 before and day 19 after the dermal patch effectiveness test.

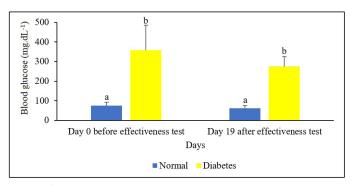


Figure 5: Bar chart to show the average fasting blood glucose of mice for each treatment before effectiveness test and after effectiveness test. The difference in letters indicates a significant difference between the treatment groups ($\alpha = 5\%$).

Public awareness about nanotechnology, especially nanoparticles, has increased drastically. In wound healing, silver nanoparticles not only have a positive impact but can have a negative impact because silver nanoparticles can be toxic to fibroblasts and keratinocytes if not in the right concentration. In addition, silver nanoparticles can accumulate in cells and cause toxic effects on these organs (Ferdous and Nemmar, 2020). From studies carried out on guinea pigs, the subchronic dose for silver nanoparticles applied to the skin is 0.1 mg/kg BW (body weight).

Silver nanoparticles were applied for 13 weeks, where every week, the silver nanoparticles were applied 5 times (Korani *et al.*, 2011).

After testing the effectiveness of the dermal patch formulated with synthetic silver nanoparticles using Plantago major L. extract, it is necessary to re-do the FBG test to ensure that STZ has an irreversible reaction, which means the damage to pancreatic cells that occurs is permanent, and mice in the diabetes group cannot return to the normal group. GDP testing was carried out on the 19th day since the dermal patch effectiveness test was started. The average fasting blood glucose level of the normal group was significantly lower (76±17 mg/dL) than the diabetes group (363±123 mg/dL) before starting the dermal patch effectiveness test. After the effectiveness of the dermal patch test, the average fasting blood glucose level of the normal group was still significantly lower (61±14 mg/dL) than the diabetes group (275±49 mg/ dL). However, the average fasting blood glucose level of the diabetic group before the start of the dermal patch effectiveness test was slightly higher (363±123 mg/dL) than after the dermal patch effectiveness test (275±49 mg/dL), but both still exceeded 150 mg/dL. Cheng et al. (2015) suggest that the regeneration of pancreatic cells by undamaged pancreatic cells may have caused the average fasting blood sugar to decrease slightly on day 19 after the dermal patch effectiveness test. Based on the analysis of wound area, epidermal reconstruction, and TIME-H data obtained from this experiment, a dermal patch formulated with silver nanoparticles using Plantago major L. with a concentration of 0.005% is effective in helping wound healing in diabetic mice because wound area, epidermal reconstruction, and TIME-H data from this dermal patch are not significantly different compared to wound dressings and silver dressings (1%) which available in the market in helping them to heal wounds on a diabetic patient. However, further tests are still needed using experimental animals with a larger size because in mice, the wound size is only 0.5 cm in diameter and the optimization of the concentration of silver nanoparticles is between 0,005% to 0,01% to get the optimal concentration. In addition, an ELISA analysis of wound tissue is also needed to observe the levels of cytokines and growth factors. Decreased levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF- can be indicators to determine the stage of healing that occurs in wounds (Younis et al., 2022) as well as the analysis of microorganisms in the





wound to find out whether there was an infection and what bacteria were found in the wounds of DM mice.

Conclusions and Recommendations

This study pioneers a novel approach by utilizing green-synthesized silver nanoparticles derived from *Plantago major* L. extract in the formulation of a dermal patch, showcasing a sustainable and environmentally friendly method for wound healing applications. Based on the analysis of the average wound area, the average epidermal reconstruction, and the average TIME-H score, the dermal patch formulated with silver nanoparticles synthesized using *Plantago major* L. extract with a concentration of 0.005% was more effective than 0.01% in helping to heal wounds in DM mice.

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Novelty Statement

The identification of the optimal silver nanoparticle concentration (0.005%) from green synthesis of *Plantago major* L. for formulating the dermal patch adds a practical and innovative element, offering a specific and valuable parameter for potential clinical applications and further differentiating this research from conventional wound care studies.

Author's Contribution

Johan Sukweenadhi: Conceptualized and designed the study, elaborated the intellectual content, performed literature search, manuscript review, manuscript revision, and guarantor.

Stefan Pratama Chandra: performed literature search, data acquisition, data analysis, statistical analysis, and manuscript preparation.

Finna Setiawan: Carried out experimental studies, performed literature search and data acquisition.

Christina Avanti and Kartini Kartini: Performed data analysis and manuscript review.

Arief Koeswanto: Performed literature search and data acquisition.

Deok-Chun Yang: Performed statistical analysis and manuscript review.

All authors read and approved the final manuscript Conflict of interest

The authors have declared no conflict of interest.

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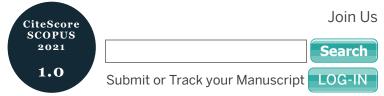


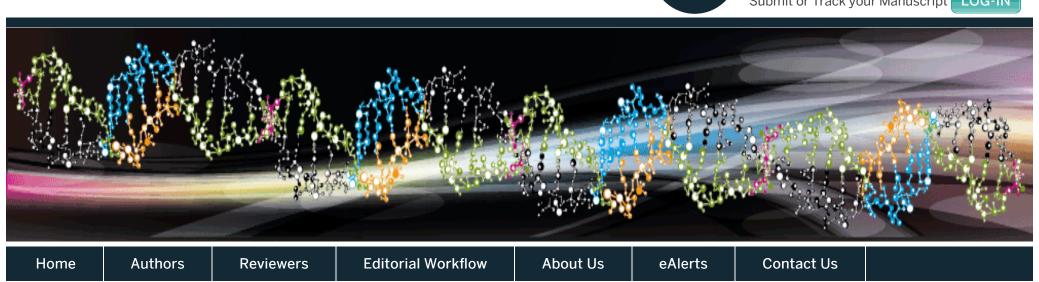


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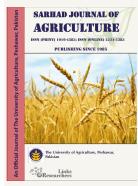
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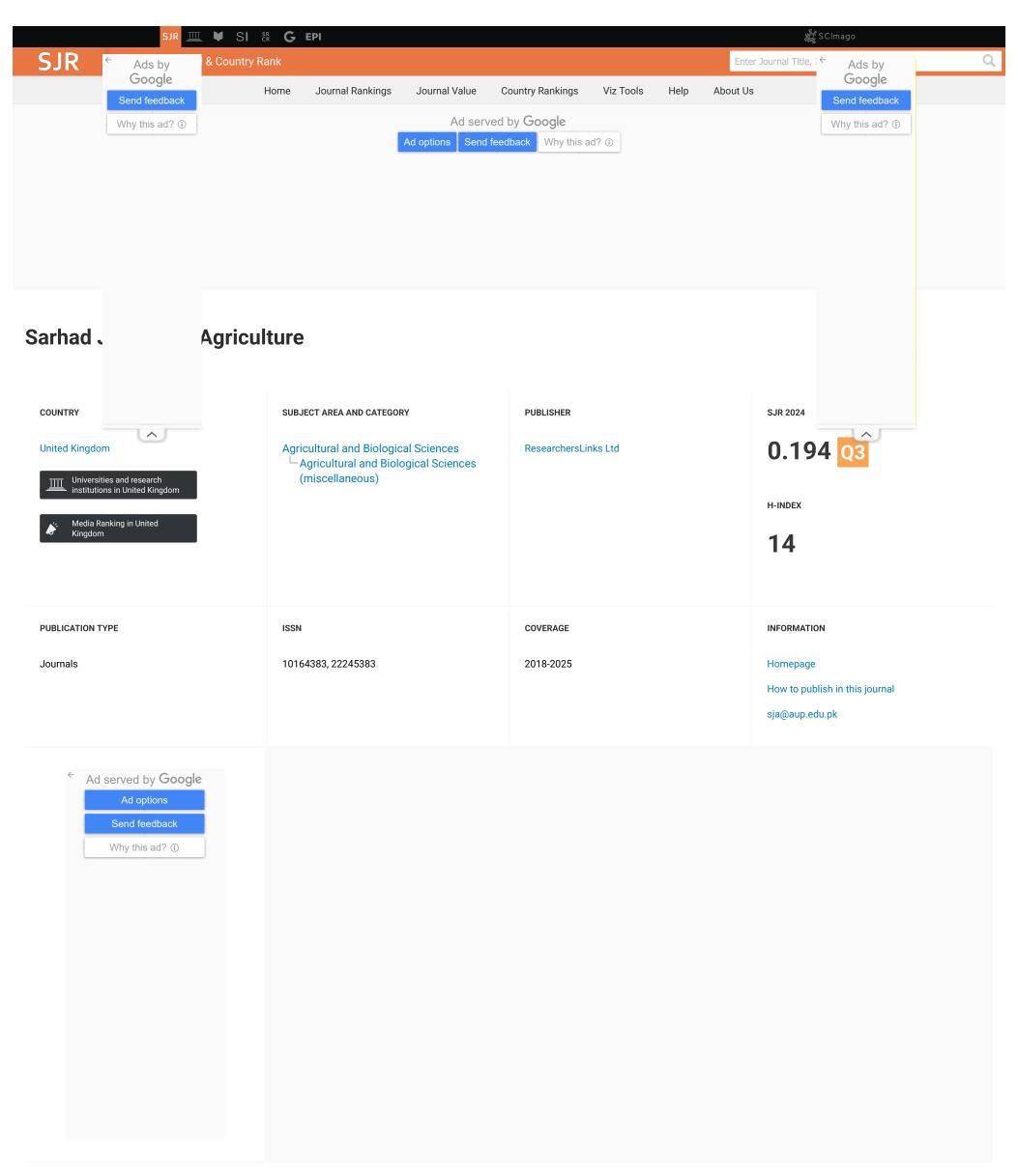
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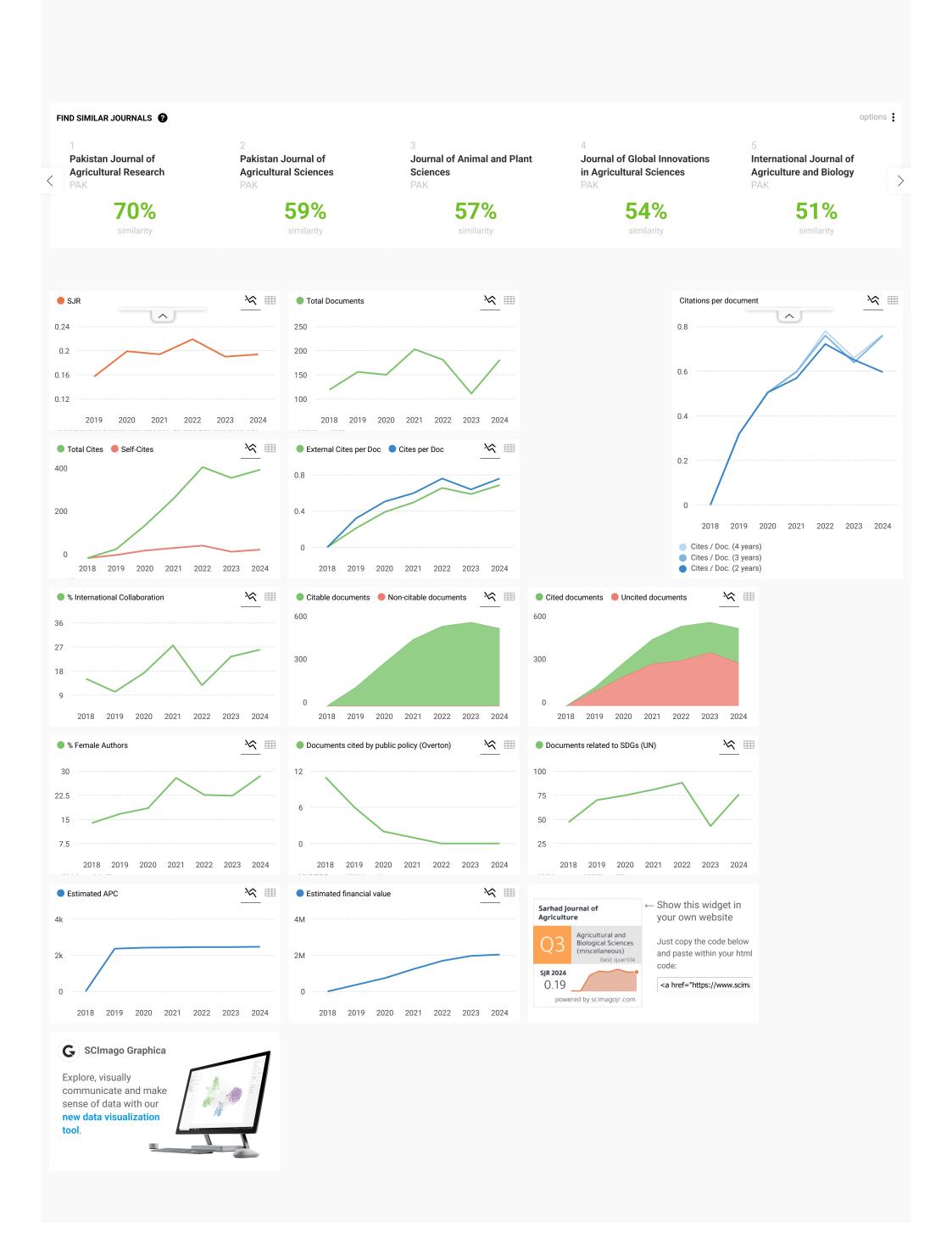
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reply



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Could someone explain?

reply reply



Melanie Ortiz 3 years ago

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update of the data. This update is sent to us around April / May every year.

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