Research Article

The Restorative Effect of Titanium Dioxide Nanoparticles Synthesized with *Origanum vulgare* L., Carvacrol, *Hypericum perforatum* L., and Hypericin Loaded in Calcium Alginate Scaffold on *Staphylococcus aureus*-Infected Ulcers in Diabetic Rats

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Abstract | In this study, the restorative effect of titanium dioxide nanoparticles (TiO2NPs) synthesized with *Origanum vulgare*, carvacrol, *Hypericum perforatum*, and calcium alginate scaffold-loadable calcium alginate on infected wounds with *Staphylococcus aureus* was investigated in diabetic rats. Eight groups of 12 rats in any group were diabetic by intraperitoneal injection of alloxan. After the rats were anesthetized, 2cm^2 wounds were created and inoculated with bacterial suspension of *S. aureus* (ATCC 12600) equivalent to the 0.5 MacFarland tube. The hydroalcoholic extracts of *Origanum vulgare*, *Hypericum perforatum*, and their active ingredients including carvacrol and hypericin, were prepared, and then TiO2N.Ps. were prepared from them using isopropoxide. The antibiotic methicillin and calcium alginate pad was also loaded as control of nanoparticles embedded in the calcium alginate pad and then the restorative effect on the wound was studied. On days 0, 3, 7, 14, and 21, hematoxylin-eosin slides (H&E) of the tissue, angiogenesis rate, and division of fibroblast cells and Masson's trichrome slides were used to determine the rate of collagenization, wound, and extracellular matrix. After the treatment, the size of the wound in the Tio2 N.P. synthesis by *Origanum vulgare*, Tio2 N.P. synthesis by carvacrol, methicillin, Tio2 N.P., and alginate pad groups of diabetic rats and alginate pad group of non-diabetic rats were determined 7.03 ± 1, 11.12 ± 1, 10.72 ± 1 ± 15.48 ± 1, 11 ± 1, and 6.35 ± 1 mm, respectively. The serum level of TNFα in the Tio2 N.P. synthesis by *Hypericum perforatum* decreased from 62 ± 1 pg/mm on day 0 to 10 ± 1 pg/mm on day 21. Medicinal plants *Origanum vulgare* and *Hypericum perforatum*, especially their active ingredients hypericin and carvacrol, reduce wound microbial load and inflammation and ultimately repair wounds in diabetic rats due to antimicrobial and anti-inflammatory activities. They can therefore be used as an effective remedy for healing wound infections in diabetics.

Keywords | Nanoparticle, Herbal plants, Diabetes, *Staphylococcus aureus*, Treatment

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INTRODUCTION

The process of wound healing and repair is accomplished through a completely complicated mechanism and is the result of undergoing various stages of repair, including inflammation, reproduction, repair, and regeneration (Eming et al., 2014; Guo Sa et al., 2010; Naveed et al., 2018). However, if this process is impaired for any reason, the process of skin repair is disturbed and the speed of wound healing is affected. Many factors affect the speed and quality of the wound healing process, including the size of the wound, local blood flow, inflammation, infection, etc. (Anderson et al., 2012; Johnson et al., 2014). In diabetes, many systems of the body, including the skin, are involved, and diabetic wounds often lead to many short- and long-term complications. According to predictions, its prevalence in the human community will increase in the future. Ischemia, neuropathy, and infection are three important pathological factors leading to complications of diabetic foot ulcers, which usually occur together (Dalla Paola et al., 2006; Vuorisalo et al., 2009). One of the major complications of diabetes is the ulcer. Often, 15% of diabetics suffer from lower extremities ulcers, of which 14-24% are amputated (Hussein et al., 2021; Setacci et al., 2009; Hicks et al., 2019). Chronic hyperglycemia is associated with impaired leukocyte function, which plays a key role in wound healing (Ekmektzoglou et al., 2006). When a wound is created, the skin is infected and allows bacteria, some of which live on its surface, to enter the bloodstream (Homaeigohar et al., 2020). Impairment in wound healing has led to a significant mortality rate worldwide, accounting for a large proportion of medical research (Ahmed et al., 2019). With the growing increase in diabetic ulcers, venous diseases, accidents, etc., the need for rapid treatment of wounds is constantly increasing. Given the high rates of this complication, finding a way to speed up wound healing is of great importance (Schreml et al., 2010). Researchers have been seeking to prevent damage to the cells due to diabetes-induced oxidative stress by using natural antioxidants (Javed et al., 2022; Al-Ani et al., 2021; Huda et al., 2022). One of the researchers' efforts aimed to find compounds to heal ulcers with the least side effects. In this regard, medicinal plants can be a good solution for wound healing (Lordani et al., 2018). Diabetic ulcers usually occur when they are exposed to infectious agents. *Staphylococcus aureus* is one of the most important infectious agents. *S. aureus* is one of the most important infectious agents that causes wound infections in diabetes. *S. aureus* is the second most common pathogen of hospital infections that is an immotile, non-spore-forming, and gram-positive coccus, which can grow in different environments (Shettigar et al., 2020). In another report, the World Health Organization has stated that 80% of people worldwide use herbal medicine in some way at the primary health care level (Solati et al., 2021; Payyappallimana, 2010; Bahmani et al., 2020; Abbasi et al., 2020; Aidy et al., 2020; Abbasi et al., 2016). Many medicinal plants contain bioactive and active antioxidant and phenolic compounds, flavonoids, etc., which have medicinal and therapeutic properties (Sytar et al., 2018). Polyphenols are a group of bioactive compounds in plants, of which more than 8,000 species have been identified (Soltanbeigi et al., 2022; Bhuyan et al., 2017; Uroko et al., 2022). Flavonoids are one of the most important categories of polyphenols. Flavonoids are polyphenol compounds that are abundantly found in plants, vegetables, and fruits. Different types of flavonoids include flavonols, flavones, flavones, anthocyanins, isoflavones, and oligomeric and polymeric forms (Bahmani et al., 2022). Flavonoids have antimicrobial, antiviral, anti-atherosclerotic, cardioprotective, anti-diabetic, antioxidant, and anti-inflammatory properties (Guven et al., 2019). Today, the use of nano-compounds is one of the best and most effective ways to treat ulcers (Negahdariet al., 2019). Antibacterial and antioxidant properties of *Origanum vulgare* and *Hypericum perforatum* have already been reported (Bahmani et al., 2019). Antibacterial and wound-repairing effects of *H. perforatum* have also been reported (Amin, 2007). Carvacrol is a major component of *O. vulgare*, which is a phenolic compound of this plant and has antimicrobial properties (Amin, 2007). Hypericin is also a compound with antibacterial activity (Kalaba et al., 2015). Both of the compounds are phenolic and flavonoid compounds with antibacterial properties. Because carvacrol and hypericin are among phenolic and flavonoid compounds of *O. vulgare* and *H. perforatum*, the restorative effects of titanium dioxide nanoparticles (TiO2NPs) synthesized with *O. vulgare*, carvacrol, *H. perforatum*, and hypericin on *Staphylococcus aureus*-infected ulcers were investigated in diabetic rats.

MATERIALS AND METHODS

Important Instruments

Origanum vulgare, *Hypericum perforatum*, carvacrol, hypericin, methicillin, microplate, plate, swab, titanium isopropoxide, alginate, calcium chloride, salt, glucometer, 2-, 3-, and 5-triphenyl tetrazolium chloride, hematoxylin-eosin stain (H & E), Masson's trichrome stain, alloxan, ketamine, xylazine, HEPES, alcohol, microtome, etc.

Studied Medicinal Plants

In this study, two medicinal plants *Origanum vulgare* (Lamiaceae family) and *Hypericum perforatum* (Hypericaceae family) were prepared and used in experiments. Besides, two standard active ingredients of the two plants, i.e., carvacrol and hypericin, which are also among the main compounds of these plants, were procured from Sigma Co. and used in experiments if necessary.

Preparation Of Hydroalcoholic Extract And Ingradient

Aerial parts of *O. vulgare* and *H. perforatum* were collected from heights of 500-550 m altitude in Mazandaran province in northern Iran (N 36 09 08 & E 53 01 22) in August 2016. Hydroalcoholic extracts obtained were filtered through Whatman filter paper grade 1 and then sterilized. First, the aerial parts of *Origanum vulgare* and *Hypericum perforatum* were collected. The plants were dried in the laboratory. 200 g of powder of each plant was mixed with ethanol 96% (Nasr Alcohol, Iran) (70:30 v/v) and distilled water. After shaking of herbal powder with water and ethanol for 4 h, the resulting solution was left in the laboratory for 24 h. After 24 h, the mixture was passed through a filter paper and distilled under vacuum conditions in a rotary evaporator (IKA® RV10) at 70 °C and 150 rpm to isolate the solvents from the extract. The concentrated extract of the plant was poured into the plate and dried.

Staphylococcus Aureus Strain

Staphylococcus aureus strain (ATCC 12600) was procured from the Iranian Research Organization for Science and Technology.

Praparation Of Tio2 N.p. .S

One ml of titanium isopropoxide solution was added to 20 ml of sterile distilled water and the mixture was slowly stirred. The solution was completely stirred for 5 h on a shaker stirrer at 50 ° C. The mixture was then stirred for 24 h at 50 °C and finally heated into a white powder, i.e., pure nanoparticle in titanium oxide solution, in a furnace at 550 °C.

Titanium Dioxide Nanoparticle

To synthesize TiO_2NPs grade 10, 10 mL of the filtered solution of the plants or compounds (*Hypericum perforatum*, *Origanum vulgare*, carvacrol, and hypericin) was added to 90 mL of a solution of 5 mM titanium isopropoxide (with pH 1.5) in a shaker Erlenmeyer at 50 °C, and after 5 h of continuous stirring, the resulting mixture was centrifuged at 12000 rpm and 4 °C for 15 min. After drying the centrifuged solution, the resulting combination was used in the laboratory and for the animal model.

Alginate Solution Praparation

To prepare the alginate solution, 100 ml of alginate gel along with 2 ml HEPES with 0.88 g NaCl and 1.25 g of alginate powder were introduced into a sterile container to a final volume of 100 ml with the addition of sterile distilled water. The Erlenmeyer was then capped with an aluminum sheet and placed on a stirrer at 60 °C temperature and a sterilized magnet for 4 h until the solution became completely homogeneous and clear. This solution was then sterilized by passing through a 0.22 µm syringe filter.

Praparation Calcium Chloride Solusion For Polymerization

To make a calcium chloride solution, 74 g of calcium chloride powder was added to 100 mL of sterilized distilled water. The calcium chloride powder was added slowly until sediment was formed so that it was visible. This solution was prepared after sterilization from the 0.22-micrometer syringe filter.

Praparation Of Depolymerizing Solution

First, a filter paper was made in the form of a 4 cm mold and placed inside glass molds. Five ml of calcium alginate gel was added to filter paper in glass molds. Then, 2 mL of saturated calcium chloride solution was added to the gel and exposed for 15 min to de-polymerize the alginate gel using a depolymerizing calcium chloride solution. The gel was then calcined by a syringe containing calcium chloride solution and the alginate pad was washed with the physiological serum. Then, 1 mL of the desired nano solution was added to the calcium alginate pad centrifugally and clockwise, and then the resulting solution was stored for 24 h at a refrigerated temperature to allow the drug to be fully loaded in the pad of interest.

Animal Model (*In- Vivo*)

Study population, sampling method, and sample size:

The protocol of this study was designed by the ethical principles approved by the international committes for the protection of animals. In this study, rats weighing 50 ± 250 g were used. The animals were placed in a clean and sterile cage and maintained under standard laboratory conditions (12-h light/12-h darkness cycles, 25 ± 3 °C, and 35-60% humidity). The food and water of rats were given routinely. The experimental protocol to deal with the rats was approved by the animal research committee of Iran's Ministry of Health and Medical Education. Twelve mice in each group were used

Diabetic Rats

In diabetic rats, alloxan was injected intra-peritoneally. After 72 h, blood glucose was measured in rats. First, the rats were inhibited, and then the blood glucose levels were measured with a glucometer card using glucometer by creating a hole in the tail with a lancet and drawing the blood. Glucose levels of rats were measured individually by a glucometer. Diabetic rats were identified and confirmed with weight loss, excessive urination, and increased blood glucose levels to at least 300 dl.

Wound Healing In Diabetic Rats

after inhibition of the rats, intraperitoneal injection of ketamine and xylazine was performed at a 3/1 ratio (for large rats) and 2/1 ratio (for small rats) to anesthetize the rats. After induction of anesthesia, the back of all rats was

shaved with an electric shaver (Moser) and then razed, and after sterilization of the skin with sterile alcohol cotton, a wound of 2 mm^2 was created by removing the skin to its complete thickness by using sterile surgical blades. Finally, the site of the wound was inoculated and infected with a sterile swab dipped in *S. aureus* bacterial suspension equivalent to a 0.5 McFarland tube. Then, the pads of each group were placed on the infected wounds and dressed with leucoplast adhesives.

Grouping Of Mice For Treatment:

Each group consisted of 12 mice. Groups include:

Group 1: Calcium alginate scaffold or pad containing TiO2NPS synthesized with hydroalcoholic extract of *H. perforatum* in diabetic rats; **Group 2:** Calcium alginate scaffold or pad containing TiO2NPS synthesized with hypericin in diabetic rats; **Group 3:** Calcium alginate scaffold or pad containing TiO2NPS synthesized with hydroalcoholic extract of *O. Vulgare* in diabetic rats; **Group 4:** Calcium alginate scaffold or pad containing TiO2NPS synthesized with carvacrol in diabetic rats; **Group 5:** Calcium alginate scaffold or pad containing TiO2NPS synthesized with methicillin in diabetic rats; **Group 6:** Calcium alginate scaffold or pad containing TiO2NPS in diabetic rats; **Group 7:** Calcium alginate scaffold or pad in diabetic rats; **Group 8:** Calcium alginate scaffold or pad in non-diabetic rats;

The area of the ulcers was recorded on days 0, 3, 7, 14, and 21 with digital colors and by shooting the process of the wound repair.

The wound healing rate was calculated according to the following formula:

Measuring The Ratio Of Wound Repair And Healing

The wound repair ratio was calculated by the following formula:

Wound healing ratio = wound size on the first day/wound size on the last day × 100

Histological investigations (Collagenization, ulcer, and extracellular matrix) were performed using hematoxylin-eosin or Masson's trichrome staining. At the end of days 0, 3, 7, 14, and 21, the blood of two rats in each group was collected, and then the rats were killed. The wound and tissue were removed to their complete thickness and placed in 10% formaldehyde for pathological studies. The tissue was embedded in paraffin, and then, using a microtome, sections with a thickness of 5-7 µ were prepared and placed on glass microscope slides. The samples were stained with hematoxylin and eosin or Masson's trichrome stain. The hematoxylin-eosin slides were used to examine the tissue composition, the degree of angiogenesis, and the division of fibroblast cells. In addition, Masson's trichrome slides were used to investigate the rate of collagenization, ulcer, and matrix. Histopathologic and histometric methods were used to measure the rate of angiogenesis by section preparation method.

Macroscopic Examination Of Wound Repair Process:

To investigate the wound repair process, the area of the wound in each group was measured using a camera installed at a fixed distance and presented in mm2.

Percentage of recovery= Percentage of ulcers – 100

Wound Healing Scoring

A Score of Wound Healing: Scoring wound repair or wound healing was performed by measuring the wound surface, the percentage of wound healing, and the time needed for the wound to close completely. Rank 1 represents the weakest grade of wound repair, and rank 7 is the highest grade.

MEASURING TNFα SERUM LEVELS

The TNFα serum level was measured by ELISA (Benderman, Germany). At the end of days 3, 7, 14, and 21, the blood sample was collected from two rats in each group, and then the rats were killed. Blood was taken from the hearts and after centrifugation at 1500-2000 rpm, blood serum was isolated and the serum level of TNFα was measured using ELISA.

RESULTS

Running the marginal model (Generalized Estimating Equations (GEE)) showed a statistical significance of time and experimental group types interaction on Average wound diameter (P<0.001) (Table 1).

Figure 1 also provides a photomicrograph of wound healing and angiogenesis stained with Hematoxylin and Eosin in days 3 to 21. Angiogenesis analysis based on the logit model and GEE showed the mutual effect of time and treatments on angiogenesis was mutually significant (p<0.001).

Histopathological staining with Mason Trichrome Color exhibited the effect of NPS on collagen density. The histopathological analysis also identified the highest collagen density in *Hypericin* Tio2 N.P treated animals (Figure 2). Collagen production by fibroblast cells increased gradually and the highest amount of collagen was documented on day 21.

Table 1: Mean± SD of wound size from day 1 to day 21

Treatments	Mean± St. dev.(mm)				
	Day 21	Day 14	Day 7	Day 3	Day 1
Tio2 N.P. synthesis by *Hypericum perforatum*	9.4±1	11.82±1.14	19.55±0.61	20±0.0	20±0.0
Tio2 N.P. synthesis by *Hypericin*	6.15±1	8.04±1.14	18.01±0.61	20±0.0	20±0.0
Tio2 N.P. synthesis by *Origanum vulgare*	7.03±1	13.74±1.14	18.88±0.61	20±0.0	20±0.0
Tio2 N.P. synthesis by *Carvacrol*	11.12±1	13.57±1.14	18.01±0.61	20±0.0	20±0.0
Methicillin	10.72±1	10.50±1.14	14.83±0.61	20±0.0	20±0.0
Tio2 N.P.	1 ±15.48	16.66±1.14	19.68±0.61	20±0.0	20±0.0
Calcium alginate pad (Diabetic rat)	11 ±1	12.38±1.14	13.54±0.61	20±0.0	20±0.0
Calcium alginate pad (Nondiabetic rat)	6.35 ±1	15.07±1.14	18.52±0.61	20±0.0	20±0.0

Table 2: Wound healing grading in different treated groups grade 1: 0-3 mm; grade 2: 6-1/3mm; grade 3: 9-1/6 mm; grade 4: 12-1/9 mm; grade 5: 1/12-15 mm; grade 6: 18-1/15 mm; grade 7: 20-1/18 mm

Group	Day 21	Wound healing grading
Tio2 N.P. synthesis by *Hypericum perforatum*	3.38 ±9.4	Grade 4
Tio2 N.P. synthesis by *Hypericin*	0.26 ±6.15	Grade 3
Tio2 N.P. synthesis by *Origanum vulgare*	1.23 ±7.03	Grade 3
Tio2 N.P. synthesis by *Carvacrol*	1.70 ±11.12	Grade 4
Methicillin	0.16 ±10.72	Grade 4
Tio2 N.P.	0.00 ±15.48	Grade 6
Calcium alginate pad (Diabetic rat)	0.00 ±11	Grade 4
Calcium alginate pad (Nondiabetic rat)	3.21 ±9.61	Grade 4

Table 3: TNF-α serum concentration in treated groups (A-H) day0-21

Groups	Period				
Day	Day0	Day3	Day7	Day14	Day21
Tio2 N.P. synthesized by *Hypericum perforatum*	62±1/41	40±1/41	15/50±0/70	13±2/82	10±1/41
Tio2 N.P. synthesized by *Hypericin*	72±2/82	42±1/41	12±1/41	11±0/00	9±1/41
Tio2 N.P. synthesized by *Origanum vulgare*	50±1/41	31/50±2/12	12/50±2/12	11±1/41	11±1/41
Tio2 N.P. synthesized by *Carvacrol*	20/50±0/70	16±1/41	14/50±0/70	13±1/41	12±0.00
Methicillin	58/50±2/12	39±2/82	16±1/41	17±1/41	12/50±2/12
Tio2 N.P.	20/50±0/70	16±1/41	15±1/41	11.50±2/12	9/50±0/70
Calcium alginate pad (Diabetic rat)	23/50±2/12	16±0/00	11/50±0/70	11±1/41	10±0/00
Calcium alginate pad (Nondiabetic rat)	70/50±0/70	50±1/41	32/50±2/12	31.50±2/12	28/50±2/12

Calcium alginate N.Ps Non-diabetic treated rats and Tio2 N.Ps treated rats respectively. The analysis also showed that Calcium alginate promoted Reepithelialisation, and increased collagen and angiogenesis in rat skin wounds. Calcium alginate had an anti-inflammatory and antibacterial effect and decreased microbial load of infectious wounds indicating a healing acceleration in Calcium alginate-treated mice. The maximum level of angiogenesis was on day 7.

Calcium alginate N.Ps and Hypericin Tio2 accelerated wound healing in multiple depths of rat's wounded skin.

O. vulgare hydro-extract contains different bioactive compounds such as carvacrol, Rosmarinic acid, and apigenin glucoside. In our study, Carvacrol was one of the major compounds of *O. vulgare* hydro-extract. Hydro-alcoholic extract of *O. vulgare* has a higher concentration of Carvacrol than hydro-extract. Carvacrol has a strong antibacterial effect. The antibacterial effect of Carvacrol Tio2 N.Ps and Hypericin Tio2 N.Ps against *staphylococcus aureus* was obvious in our study. The anti-S. aureus effect of Tio2 synthesized by Hydro-alcoholic extract of *H. perforatum* and Hypericin were also significant. It was demonstrated that a bioactive compound named imanine available in Hydro-alcoholic extract *H. perforatum* is responsible for potential activity against *S.aureus*.

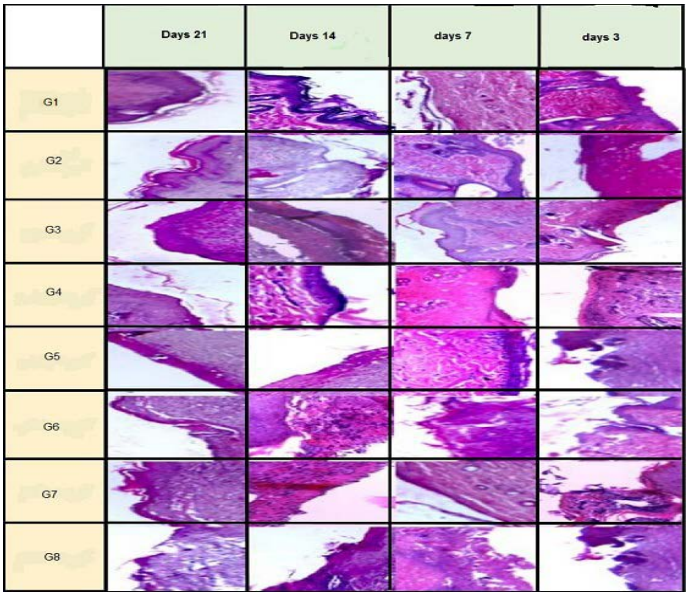

Figure 1: Wound healing steps in different treatment groups (day 3-21) - Grage 1-8 (H&E staining)

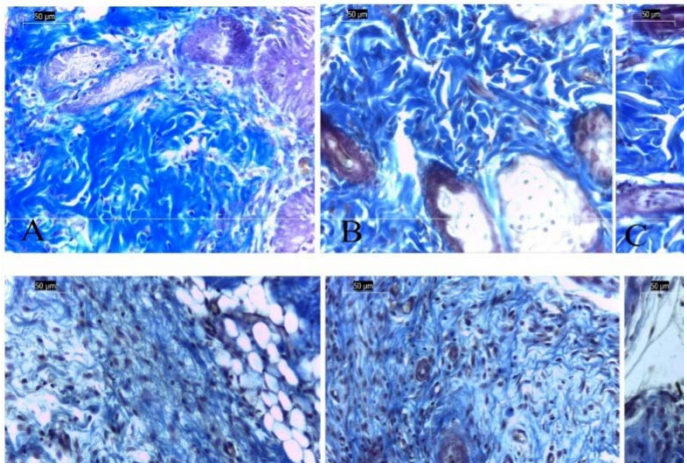

Figure 2: A: Tio2 N.P. synthesis by *Hypericin* ؛ **B:** Calcium alginate pad (Nondiabetic rat)؛ **C**: Tio2 N.P. synthesis by *Origanum vulgare*; **D**: Tio2 N.P. synthesis by *Hypericum perforatum*; E: Methicillin; **F:** Calcium alginate pad (Diabetic rat); **G:** Tio2 N.P. synthesis by *Carvacrol*; **H:** Tio2 N.P. (Highest to lowest collagen density in different NPs treated rat A-H)

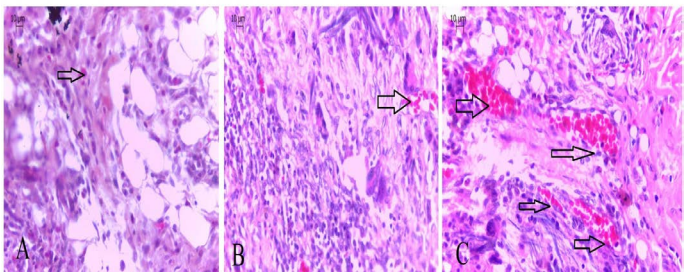

Figure 3: H&E staining Photomicrograph of angiogenesis (groups A-C) - **A:** Hypericin Tio2 N.P. ؛ **B:** Calcium alginate pad (Non-diabetic rat) ؛ **C**: Tio2 N.P

Histopathological analysis of H&E staining showed the highest angiogenesis in Hypericin Tio2 N.Ps treated rats, Because of the antibacterial effect of Carvacrol and Hypericum, the green synthesized nanoparticles by these compounds could be effective against microbial infections The main bioactive compound of *O. Vulgare* and *H. perforatum* was Carvacrol and Hypericin.

The wound diameter in rats treated with *H. perforatum* Tio2 N.Ps was reduced to 9.4±.3.38 on day 21 from 20mm on day 0. The wound diameter in rats treated with *Hypericin* Tio2 N.Ps was reduced to 6.15±0.26 on day 21. It should be mentioned that wound diameter for *O. vulgare* Tio2 N.Ps, *Carvacrol* Tio2 N.Ps, Methicillin, Tio2 N.Ps, Calcium alginate pad (Diabetic rat), and Calcium alginate pad (Nondiabetic rat) were7.03±1.23, 11.12±1.70, 10.72±0.16, 15.48 ± 0.00, 11±0.00, 9.61±3.21 respectively. Serum level of TNF-α in *H. perforatum* and *Hypericin* Tio2 N.Ps treated mice reduced from 62±1.41 and *72±2.82 pg/mL on day 0 to 10±1.41 pg/mL and 9±1.41 pg/mL in day 21.* TNF-α *serum level were 9.41±1.41 pg/mL, 11±1.41 pg/mL, 12±0.00 pg/mL, 12.5±2.12 pg/mL, 9.5±0.7 pg/mL, 10±0.00 pg/mL, 28.5±2.12 pg/mL for O. vulgare* Tio2 N.Ps, *Carvacrol* Tio2 N.Ps, Methicillin, Tio2 N.Ps , Calcium alginate pad (Diabetic rat) and Calcium alginate pad (Non diabetic rat) in day 21 respectively.

DISCUSSION

Infectious and non-infectious diseases are common and researchers are looking for ways to treat them (Darvishi et al., 2015; Ghazimirsaeid et al., 2014; Darvishi et al., 2017; Pirhadi et al., 2021; Darvishi et al., 2020; Moudi et al., 2012; Darvishi et al., 2016; Barary et al., 2021).

Different steps are actively involved in the wound healing process, which includes inflammation, proliferation, and remodeling of the wounded tissue. The wound healing process initiates with platelet aggregation and clotting factor release leading to fibrin clot deposition (Nikpasand et al., 2019). Nostro et al showed that Origanum vulgare essential oil and carvacrol had an inhibitory effect against S.aureus. In their study the MIC of Origanum vulgare and carvacrol were 0.062% and 0.015% v/v. their study also showed the inhibitory influence of *H. perforatum* on different types of *S.aureus* (Nostro et al., 2007). A recent study showed a promising result of Hypericin against methicillin-resistant *S. aureus* (MRSA) wound infection. Chan et al in the study demonstrated that antibacterial photodynamic therapy mediated by Hypericin has a lower minimum bactericidal concentration (MBC) value (0.625–10 μM) against MRSA compared to theophobia a (Pa) or methylene blue (MB). Their histological analysis showed Reepithelialisation and wound healing in the Hy-PDT group compare to Pa or MB. They showed that Hy-medi-

ated PDT is an effective therapeutic option against MRSA (Chan et al., 2021). By their study, our results also showed the effectivity of Hypericin on the wound healing process in S.aureus infected skin. TiO2 NPs synthesized by Hypericin had the best wound healing compared to other agents. Our histological analysis also showed that the highest collagen production was in the rats treated with Hypericin TiO2 NPs. TiO2 yields an antibacterial effect through UV radiation exposure because of its oxidation properties (Bono et al., 2021). TiO2 can attach to the pathogen's surfaces and leading to an antibacterial effect by absorbing the radiation. Different types of NPs have an antibacterial effect based on their surface area to volume ratio (Kumaravel et al., 2021). A recent study conducted by Khaksarian and his COLLEAGUES SHOWED TiO2 NPs synthesized by O. Vulgare, H. perforatum, carvacrol, and Hypericin, and TiO2 had MIC of 250, 62.5, 250, and 250, and 500 µg/mL, respectively (Khaksarian et al., 2022). Nanofibers with H. perforatum (HPO) loaded PCL/Ge blends to promote self-repair of diabetic wounds in Wistar albino male rats (Guleken et al., 2021). In diabetic wounds, different types of inflammatory cytokines are released in the wound microenvironment. The most important cytokines include TNF-α, transforming growth factor, and interleukins (Guleken et al., 2017). Hypericum perforatum oil could decrease the Total oxidant level (TOS) in rats and reduce the tumor necrosis factor-alpha (TNF-α) levels (Farcas et al., 2019). HPO dressing is more efficient than Aloe Vera to heal skin wounds in the inflammation phase (Guleken et al., 2017). HPO extract contains major bioactive compounds such as tannins, hyperin, hypericin, and hyperforin. Hypericin is an anti-inflammatory agent and hyperforin poses antibacterial effects (Saddiqe et al., 2010). Our results also showed a promising effect of Hypericum perforatum and Hypericin NPs on wound healing in diabetic rats.

HPO also seems to reduce lipid peroxidation leading to cell necrosis deceleration and vascularity improvement (Silva et al., 2008; Yadollah-Damavandi et al., 2015). In agreement with this conclusion, H&E staining analysis in our experiment showed the highest amount of angiogenesis in diabetic rats.

CONCLUSIONS

The histological results of our study demonstrated that TiO2 NPs synthesized by the green synthesis process and their active compounds loaded on calcium alginate scaffold, Especially, hypericin TiO2 NPs accelerated the healing process including collagen formation, angiogenesis, and proinflammatory cytokines inhibition in all depth of infectious wounds in rat skin. Based on the result of this study we concluded that plant-based nanoparticles especially hypericin TiO2 NPs are a suitable alternative wound dressing for diabetic infections wounds.

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CONFLICTS OF INTEREST

The authors declared no competing interests.

ETHICAL CONSIDERATION

Ethical issues (including Plagiarism, data fabrication, double publication, etc.) Have been completely observed by the author.

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None.

AUTHORS' CONTRIBUTION

All authors contributed equally to the manuscript.

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