*Open Veterinary Journal*, (2024), Vol. 14(6): 1358-1369
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
*Research Article*
DOI: *10.5455/OVJ.2024.v14.i6.4*

Submitted: 02/02/2024 Accepted: 21/05/2024 Published: 30/06/2024

# Effect of pomegranate peel with/without autologous bone marrow on healing of acute cutaneous wounds in alloxan-induced diabetic rabbits

Fahd A. Al-Sobayil[1], Meshal M. Alsupail[2] and Mohamed Tharwat[1,3]*

[1]*Department of Clinical Sciences, College of Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia*
[2]*National Unified Procurement Company (NUPCO), Riyadh, Saudi Arabia*
[3]*Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*

**Abstract**

**Background:** Healing of bum wounds is commonly associated with many complications. Every year various new repair materials are developed and experimentally used for treating burn wounds. Humans with diabetes mellitus usually suffer from chronic wound healing. Vascular, neuropathic, immune function, and biochemical abnormalities each contribute to the altered tissue repair. One underlying factor that accompanies all diabetic ulcerations is poor vascular flow, a circumstance that impedes proper wound healing. Numerous studies have highlighted the importance of adequate vascular sufficiency and vessel proliferation in tissue repair and the lack thereof in diabetic wound healing. Other studies have looked at whether disarrayed capillary remodeling and maturation of vessels might play a role in impaired diabetic wound healing.

**Aim:** This investigation has been planned to report the influence of treatment with a mixture of both the powder of pomegranate peel (PP) accompanied with an autologous bone marrow (BM) on the cure of burn injuries in experimentally induced diabetic rabbits.

**Methods:** Alloxan monohydrate has been applied to create diabetes in 50 rabbits. Then in each rabbit, two deep second-degree burn wounds were experimentally created. The animals were then divided randomly into 5 treatment sections: non-treatment controls (C1), treated with an available commercial powder for wound (C2), treatment with powder of PP, treatment with alone BM, and the final group treated with PP powder with bone marrow (PPBM). The speed of wound closure and the histopathological changes during healing were measured. The levels of the biomarkers of rabbit platelet-derived growth factor AA (PDGF-AA) and rabbit protease-activated receptor 1 (PAR-1) were measured on days 0, 4, 8, and 12.

**Results:** Wound healing was markedly more rapid in all the treatment groups versus the control non-treated group. Interestingly, a rapid wound cure was significantly observed in the PPBM group versus the other treatment ones. The histological assessment clarified a significant elevation in the fibroblast and collagen scores in the PPBM group versus the other sections. In addition, there were significant increases in the serum levels of the biomarkers PDGF-AA and PAR-1 among groups.

**Conclusion:** Dependent on the results of current research, it can be concluded that both PP powder with BM PPBM significantly accelerate the healing process of burn wounds in experimentally induced diabetic rabbits.

**Keywords:** Alloxan, Diabetes mellitus, Bone marrow, Rabbit, Wound healing.

## Introduction

Skin burns appear due to several etiologies; of them, electricity, heat, chemicals, friction, or radiation. They are generally categorized into superficial, partial superficial, partial deep, and full skin thickness burns. The intensification of the burns relies primarily on the extent and depth of the wound but also on position, age, and accompanied systemic disorders (Burgess *et al*., 2022). The major concerns for survivors in most patients are those associated with the problems of healing and scarring formation. Even after healing, later complications such as contractures and hypertrophic scarring are common in burn wounds. In fact, these complications have always been the main challenge facing practitioners. Therefore, many new repair materials are annually developed and experimentally used for treating burn wounds (Dziewulski, 1992).

Severe firing wounds are the most invasive resulting in high morbidity and mortality rates. Based on the records of the WHO, it is found that annually almost 11 million experience burn injuries, 180,000 of whom collapse (Markiewicz-Gospodarek *et al*., 2022). During the past 20 years, different firing animal models have been improved to elucidate the pathophysiology and

***Corresponding Author:** Mohamed Tharwat. Department of Clinical Sciences, College of Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia. Email: *atieh@qu.edu.sa*

potential treatment interventions; of them, rabbits are an appropriate animal model (Abdullahi *et al*., 2014).

Diabetes mellitus (DM) is one of the essential contributors to chronic wound healing issues as almost 25% of all diabetic patients often suffer from lower extremity amputation, with the following high financial and psychosocial drawbacks. Hyperglycemia encourages the establishment of biofilms and, therefore, making wounds in diabetic patients hard to treat (Burgess *et al*., 2021). The pathophysiologic relationship between diabetes and impaired healing is complex. Vascular, neuropathic, immune function, and biochemical abnormalities each contribute to the altered tissue repair. Although curing of these wounds, which includes strict glucose monitoring and wound care, the outcome for their healing is completely unfavorable (Greenhalgh, 2003). In addition, one underlying factor that accompanies all diabetic ulcerations is poor vascular flow, a circumstance that impedes proper wound healing. Numerous studies have highlighted the importance of adequate vascular sufficiency and vessel proliferation in tissue repair and the lack thereof in diabetic wound healing (Prompers *et al*., 2008). More studies have looked at whether disarrayed capillary remodeling and maturation of vessels might play a role in impaired diabetic wound healing (Okonkwo and DiPietro, 2017).

Pomegranate (*Punica granatum*) belongs to the family *Punicaceae* and its peel has been implemented in traditional medicine. Pomegranate peel has the advantages of promoting hemostasis, effectiveness in restraining of the intestine, and beneficial effects in parasite killing (Tang *et al* 2011). It has been reported that pomegranate oil has improved wound-healing properties (Uzunhisarcıklı and Yerer, 2021). It has been reported also that pomegranate peels enhance cutaneous wound healing (Murthy *et al*., 2004). Powder of standardized pomegranate extract accelerated also the healing of deep second-degree burn wounds (Lukiswanto *et al*., 2019). The usage of pomegranate peel polyphenol gel is reported to be a beneficial method for treating wound disorders associated with diabetes (Yan *et al*., 2013).

Bone marrow (BM) is a spongy substance found in the center of the bones and is considered as an important source of stem cells and other substances. It was reported that BM-derived stem cells may be involved in the acceleration of wound healing (Wu *et al*., 2007) including burn injury (Xue *et al*., 2013). It was recently reported also that transplantation of BM cells pre-activated with sodium nitroprusside enhances acute wound curing in rabbits (Fatima and Saleem, 2023). Parelle, enhancement of chronic wound curing by pre-activated BM cells with sodium nitroprusside in rabbits has been reported (Fatima *et al*., 2022). It was found also that BM-derived mesenchymal stem cells are the hopeful curative choice for the therapy of diabetic foot wounds (Dama *et al*., 2023).

The effects of pomegranate peel in combination with BM on burn wound healing have not been reported. Therefore, the current study was to investigate the effect of PP with or without autologous BM on the curing of acute skin wounds in alloxan-induced diabetic rabbits. Data of this paper has been partially presented at the 28th Annual Meeting of the Wound Healing Society, SAWC-Spring/WHS Joint Meeting: Georgia World Congress Center, Atlanta, Georgia, USA, April 13-17, 2016.

## Materials and Methods

### *Animals*

Fifty adult male New Zealand rabbits (weight 2.86 ± 0.24 kg) were divided randomly into five treatment groups: (1) non-treatment control (C1), (2) treatment of wounds with a commercial powder (C2), (3) treatment with the powder of pomegranate peel (PP), (4) curing with BM alone, and (5) powder of PP powder with BM (PPBM). The animals were housed in a room containing cages with a temperature range of 20 °C–25°C and humidity of 65%–70%. During the study, the animals were fed with usual rabbit pellets and watered through tap water.

### *Induction of diabetes*

Alloxan monohydrate (AM) (Sigma, Saint Louis, MO, USA) was used to induce DM in rabbits. First, AM was dissolved in sterile normal saline to make a solution of 5% (W/V), and followed by an immediate intravenous injection of 100 mg/kg through the marginal ear vein over a period of 2 minutes using a 25-gauge butterfly catheter (Wang *et al*., 2010). To prevent hypoglycemic shock, a solution of glucose 20% was given via water *ad libitum* for 1–2 days.

### *Induction of burn wound*

Each rabbit was first sedated with xylazine (5 mg/kg IM) and then anesthetized with ketamine (25 mg/kg IM). The skin over the ventrolateral back was shaved and then disinfected with betadine hydrochloride. A round hot plate (2 cm diameter) was used to induce a deep second-degree burn wound on both back sides. The hot plate was placed 10 seconds on the skin with a similar pressure.

### *Treatment of groups*

After 24 hours, the program of treatment was started. Rabbits in group C1 were left as a control without any medication. Rabbits in group C2 were treated with commercial wound powder (WOUNDJAT, Saudi Arabia) once a day. For each rabbit, both wounds were covered with the powder. Rabbits in group PP were treated with PP once a day where the wound was covered with the PP powder. Rabbits in group BM were treated with only an injection of autologous BM (0.5 ml in each wound) 3 days later after the wound induction. The autologous BM was collected from the left or right tibia. In group PPBM, rabbits were treated with PP plus fresh autologous BM samples. Three days after the wound induction, 0.5 ml of BM sample was injected just below the formatted crust of each wound (Fig. 1).

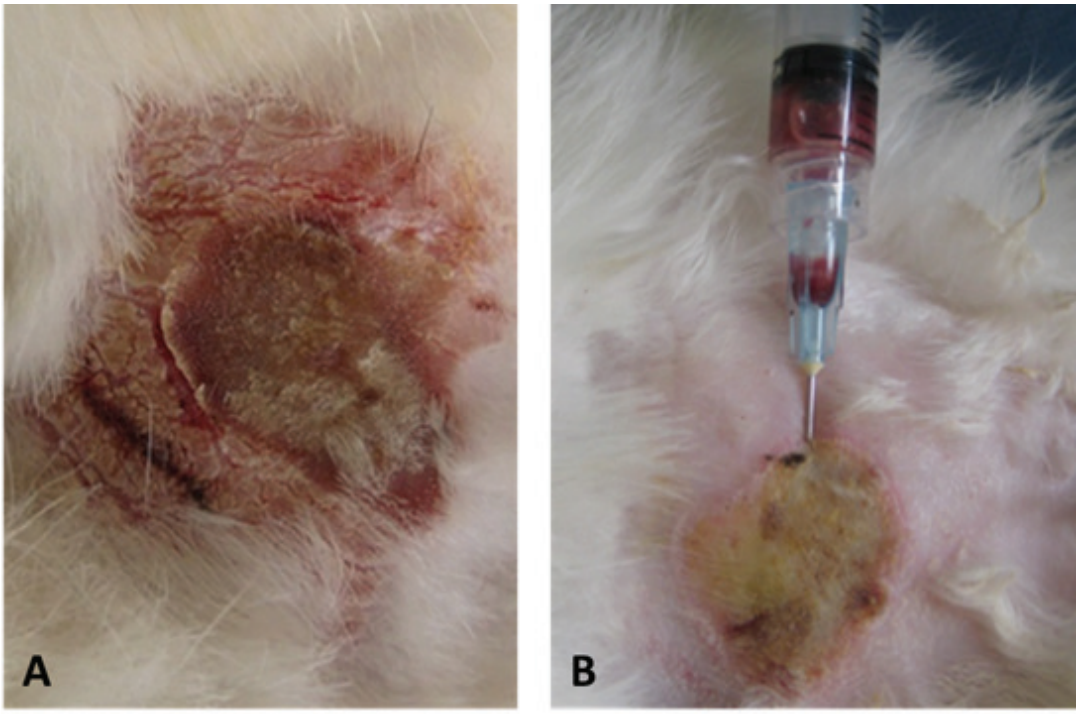


**Fig. 1.** (A): Formatted crust. (B): Bone marrow sample (0.5 ml) was injected just below the formatted crust of each wound.

A fresh BM sample was surgically harvested by anesthetizing the rabbit using a combination of xylazine 5 mg/kg IM with ketamine 25 mg/kg IM. The skin incision was made in the proximal medial side of the tibia. After the elevation of the periosteum, a hole was made in the bone using an electric drill with a 0.2 mm drill bit. About 1 ml of BM was harvested and immediately injected into both wounds just under the formatted scab (0.5 ml in each side) in individuals belong to BM and PPBM groups. BM samples were also harvested from rabbits belonging to the other three groups but were discarded.

***Evaluation of wound healing***

The healing of the wounds was evaluated grossly, histopathologically, and biochemically. The wound in the left side of the back was used for the gross evaluation. The gross parameters measured were composed of the degree of edema, hyperemia, and epithelization. These were assessed according to the following scoring system: 0 none, 1 minimal, 2 mild, and 3 maximum (Schlager *et al*, 2000 a,b). The wound closure was evaluated daily by measuring the diameter of the wound. The wound in the right side was used to perform the histopathological changes. The biopsy specimens were collected from the burn wound on days 0, 4, 8, and 12 from the following locations 12, 3, 6, and 9 O'clock, respectively. The specimens were placed in 10% formaldehyde and fixed in paraffin, then 4 µm sections were stained with Hematoxylin and eosin (H&E), Masson's trichrome stain, and evaluated under the light microscope.

***Scoring of wound healing***

The parameters of histopathological evaluation were epithelization, vascularization, collagen, and fibroblasts. These parameters were evaluated based on the modified scoring system 0–3 (0 = none, 1 = minimal, 2 = mild, 3 = evident) (Schlager *et al*., 2000 a,b). Abramov's histological scoring system was used for scoring epithelialization, fibrosis, angiogenesis, and collagen level (Table 1) (Abramov *et al*., 2007).

***Biochemical evaluation of wound healing***

The biochemical evaluation of the wound was performed by measuring the concentrations of wound biomarkers in the serum. Blood samples were taken from the ear vein of each rabbit on days 0, 4, 8, and 12. The blood was centrifuged at 3,000 rpm for 10 minutes and the serum was transferred to numbered tubes and stored at −20°C for 2 weeks. Commercially available ELISA kits were used to determine the levels of the following biomarkers: rabbit platelet-derived growth factor AA (PDGF-AA) (MyBioSource, California, USA) and rabbit protease-activated receptor 1 (PAR-1) (MyBioSource, California, USA).

***Statistical analysis***

Data were analyzed using SAS software and the significance level was fixed at $p < 0.05$. A repeated measures design and *Tukey* test were used to assess the statistical significance of the quantitative parameters.

**Table 1.** Histological scoring system for scoring epithelialization, fibrosis, angiogenesis, and collagen level (Abramov *et al*., 2007).

| | Epithelialization | Angiogenesis | Fibroblasts | Collagen | Macrophages/lymphocytes |
|---|---|---|---|---|---|
| a: control | 1 | 3 | 3 | 1 | 3 |
| b: curcumin 2% | 1 | 3 | 3 | 1 | 2 |
| c: Thc 2% | 2 | 2 | 3 | 2 | 2 |
| d: Thc 3% | 2 | 2 | 3 | 2 | 2 |
| e: Thc 5% | 2 | 2 | 2 | 3 | 2 |
| f: glucosyl-Thc 2% | 2 | 2 | 2 | 2 | 1 |
| g: glucosyl-Thc 3% | 2 | 1 | 2 | 3 | 1 |
| h: glucosyl-Thc 5% | 3 | 0 | 0 | 3 | 1 |

Abramov's histological scoring system for scoring epithelialization, fibrosis, angiogenesis, and collagen level. Abramov's system assesses each parameter independently, giving each a score of 0–3, as follows:

- Collagen: 0= none, 1= scant, 2= moderate, 3= abundant.
- Epithelialization: 0= none, 1= partial, 2= complete but immature/thin, 3= complete and mature.
- Angiogenesis: 0= none, 1=#5 vessels per hPF, 2=6–10 vessels per hPF, 3=.10 vessels per hPF.
- Fibrosis: 0= none to minimal fibroblasts, 1= few fibroblasts, 2= more fibroblasts, 3= predominantly fibroblasts.
- The number of macrophages was scored, as: 1=0–25 macrophages, 2=26–50 macrophages, 3=.51 macrophages.

(glucosyl-THC): glucosyl-conjugated tetrahydrocurcumin; (HPF): high-power field; (THC): tetrahydrocurcumin.

**Table 2.** Mean (±SE) time of wound closure for each group.

| Group | Number | Time of wound closure (days) | Complications |
|---|---|---|---|
| **C1** | 10 | 25.2 ± 2.6 [a] | Septic infection ($n$ = 2) |
| **C2** | 10 | 17.2 ± 2.3 [b] | None |
| **PP** | 10 | 14.3 ± 1.4 [c] | None |
| **BM** | 10 | 16.5 ± 2.1 [b] | Septic infection ($n$ = 1) |
| **PPBM** | 10 | 12.2 ± 1.1 [d] | None |

**(C1):** control; **(C2):** commercially wound powder; **(PP):** pomegranate peel powder; **(BM):** bone marrow alone, **(PPBM):** pomegranate peel powder with bone marrow.

***Ethical approval***

Animals were treated according to the *Laboratory Animal Control Regulations* of Qassim University which are correlated well with the recommendations of *Laboratory Animals Control Guidelines* of the national institutes of animal health (USA, 86–23, 1996).

## Results

All except 3 rabbits survived; two rabbits in group C and one in group BM had septic infection. The time taken for complete wound closure in the animals of each group is summarized in Table 2. The shortest time was found in the group of PPBM (12.2 ± 1.1 day) while the longest was in group C1 (25.2 ± 2.6 day) with a statistically significant difference between them ($p < 0.05$). In the group of PP alone, time was 14.3 ± 1.4 day compared to 16.5 ± 2.1 day in the group of BM alone ($p < 0.05$). There was no significant difference between groups C2 and BM (17.2 ± 2.3 day versus 16.5 ± 2.1 day) ($p > 0.05$).

Figure 2 shows healing of wounds healing at days 0, 4, 8, and 12 in control groups (C1 and C2) and groups treated either with PP, BM, or PPBM. Wound healing was significantly more speed in all the treatment groups versus the control groups. Contrary, rapid wound curing was significantly occurred in PP and PPBM groups versus the other treatment groups. At day 0, wound sections showed scant collagen (Masson's trichrome stain ×400), inflammatory cells score -3, fibroblasts score 0 and angiogenesis score 0 (H&E stain × 400). At day 8, wound sections showed inflammatory cells score-1, fibroblast score-3 and angiogenesis score-2 (H&E stain × 400), inflammatory cells score-2, fibroblasts- score- 2, angiogenesis score-3 (H&E stain × 400), and inflammatory cells score-2, fibroblasts score-3, moderate collagen and full epithelium (Masson's trichrome stain × 100). At day-21, wound sections showed abundant collagen score-3 (Masson's trichrome stain × 100), inflammatory cells score-1, angiogenesis score-0, fibroblasts score-1 full epithelium (H&E stain × 400) (Fig. 3).

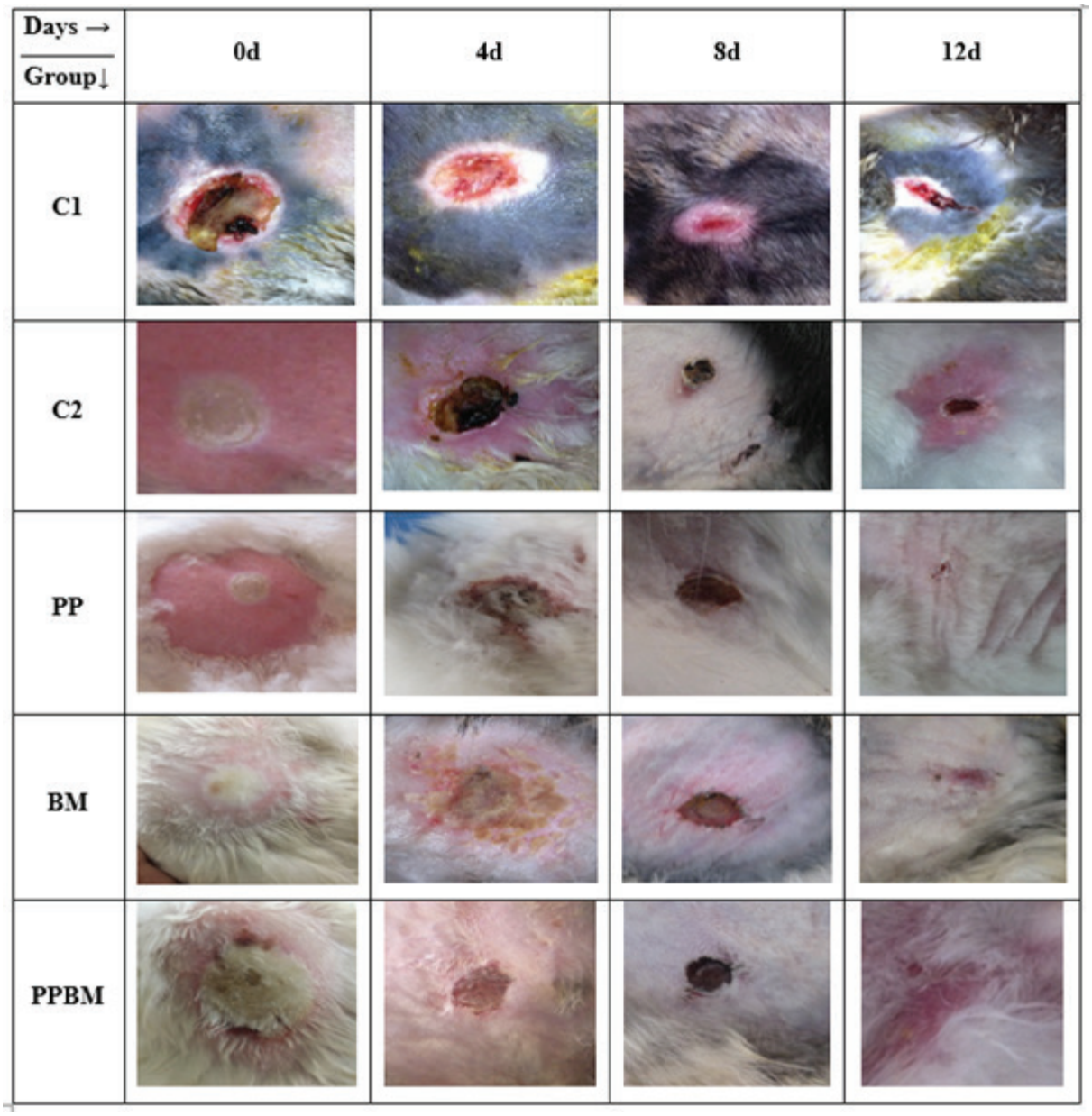


**Fig. 2.** Images of wound healing at days 0, 4, 8, and 12 in groups of controls (C1 and C2), pomegranate peel (PP), bone marrow (BM), and pomegranate peel with bone marrow (PPBM).

Table 2 shows histopathological scoring for the C1, C2, PP, BM, and PPBM groups on days 4, 8, and 12. The histological assessment revealed marked elevations in the fibroblast and collagen scores in the 2 groups of PP and PPBM versus others. This might explain the speed of wound closure in these two groups compared to the others (Table 3; Fig. 3). At day 4, compared to groups C1, C2, and BM, angiogenesis was significant in groups of PP and BMPP ($p < 0.05$). Similarly, collagen formation was significant in groups of PP and BMPP ($p < 0.05$) compared to groups of controls (C1 and C2) and BM group. However, fibroblast formation was significant in 3 groups (PP, BM, and PPBM) versus control groups (C1 and C2) ($p < 0.05$). At day 8 and day 12, compared to groups C1, angiogenesis was significant in all other groups (C2, PP, BM, PPBM) ($p < 0.05$). Similarly, fibroblast and collagen formation were significant in all other groups compared to group C1 ($p < 0.05$) (Table 3; Fig. 3). Significant increases in the concentration of PDGF-AA in all groups on days 8 and 12 compared to day 0 (Fig. 4). The levels of PDGF-AA were significantly higher in all 5 groups versus the control group on days 4, 8, and 12. The levels of PDGF-AA were significantly elevated in PP and PPBM

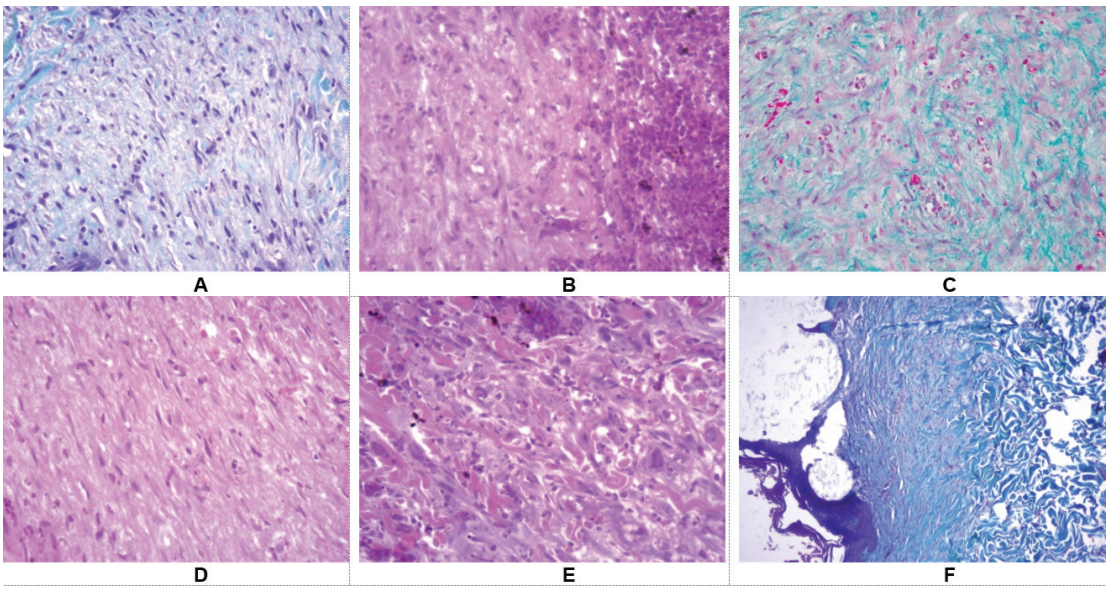


**Fig. 3.** Histopathological scoring for inflammatory, angiogenesis, fibroblasts, and collagen in alloxan-induced diabetic rabbits. At day 0, wound sections show scant collagen (Masson's trichrome stain × 400) (A), inflammatory cells score-3, fibroblasts score-0 and angiogenesis score-0 (H&E × 400) (B) and Scant collagen score-1, inflammation score-3 (Masson's trichrome stain × 400) (C). At day 8, wound sections show inflammatory cells score-1, fibroblast score-3, and angiogenesis score-2 (H&E × 400) (D) and inflammatory cells score-2, fibroblasts- score- 2, angiogenesis score-3 (H&E × 400) (E). At day 21, wound sections show abundant collagen score-3 (Masson's trichrome stain × 100), Inflammatory cells score-1, angiogenesis score-0, fibroblasts score-1 full epithelium (H&E × 400) (F).

**Table 3.** Mean (±SE) histopathological scoring for the groups at days 4, 8, and 12.

| Days | Groups | Angiogenesis | Fibroblasts | Collagen |
|---|---|---|---|---|
| **4** | C1 | 0.7 ± 0.4 | 0.4 ± 0.2 | 0.3 ± 0.2 |
| | C2 | 0.9 ± 0.3 | 0.7 ± 0.3 | 0.4 ± 0.2 |
| | PP | 1.4 ± 0.4* | 1.1 ± 0.4* | 0.9 ± 0.2* |
| | BM | 1.1 ± 0.2 | 1.0 ± 0.3* | 0.5 ± 0.3 |
| | PPBM | 1.4 ± 0.3* | 1.3 ± 0.4* | 0.9 ± 0.2* |
| **8** | C1 | 0.8 ± 0.4 | 0.7 ± 0.3 | 0.6 ± 0.3 |
| | C2 | 1.1 ± 0.2* | 1.0 ± 0.3* | 0.9 ± 0.3* |
| | PP | 2.2 ± 0.3* | 2.8 ± 0.1** | 2.2 ± 0.3* |
| | BM | 2.2 ± 0.5* | 2.0 ± 0.4* | 1.0 ± 0.4* |
| | PPBM | 2.7 ± 0.3** | 2.9 ± 0.1** | 2.8 ± 0.1** |
| **12** | C1 | 1.4 ± 0.4 | 1.4 ± 0.5 | 1.7 ± 0.6 |
| | C2 | 2.3 ± 0.4* | 2.1 ± 0.3* | 2.3 ± 0.3* |
| | PP | 2.8 ± 0.1** | 2.5 ± 0.0* | 2.6 ± 0.3* |
| | BM | 2.5 ± 0.3* | 2.6 ± 0.3* | 2.1 ± 0.5* |
| | PPBM | 2.9 ± 0.1** | 2.7 ± 0.0* | 2.8 ± 0.1** |

**C1**, control; **C2**, commercially wound powder; **PP**, pomegranate peel powder; **BM**, bone marrow alone; **PPBM**, pomegranate peel powder with bone marrow. *$P$<0.05; **$P$<0.01.

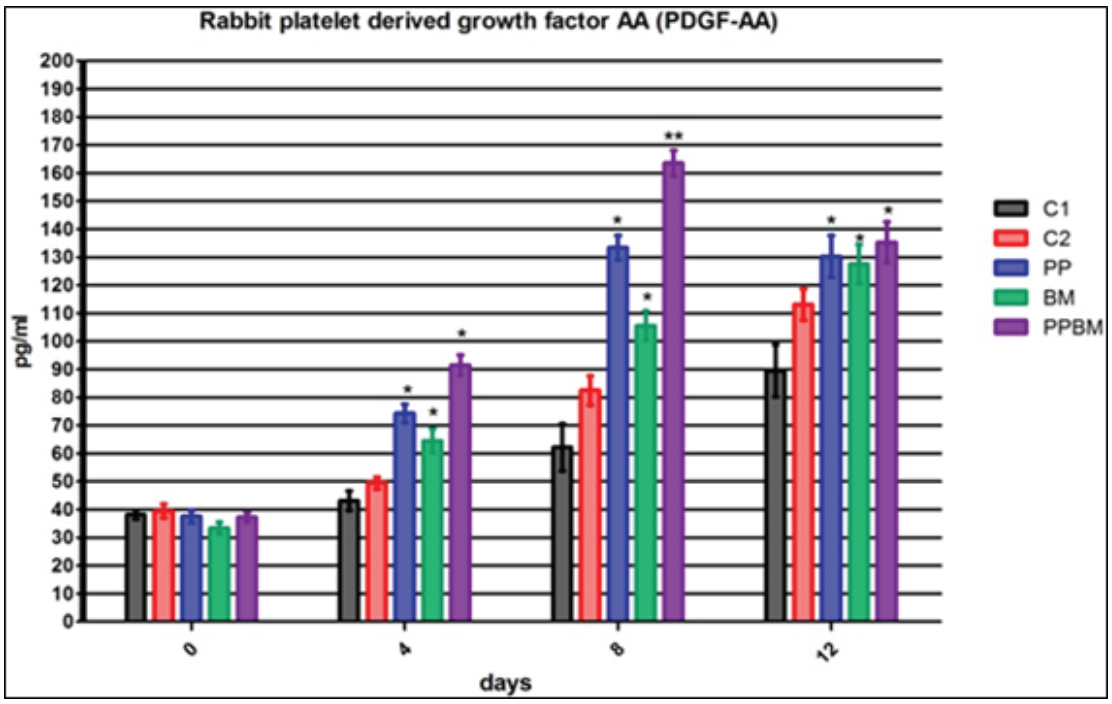


**Fig. 4.** Mean (±SE) level of concentration for rabbit platelet-derived growth factor AA (RPDGF-AA) at days 0, 4, 8, and 12.

groups in comparison to the other groups on days 4 and 8. The levels of PAR-1 significantly showed increases on days 4 and 8 in all the groups versus to the control (Fig. 5) and then its level decreased on days 12 in all groups except the C1 group. On day 12, the levels of PAR-1 were significantly higher in the control group compared to the other groups.

## Discussion

To the authors' information, this is the first study showing the influence of PP powder with or without an autologous BM on the curing of acute skin wounds in alloxan-induced diabetic rabbits.

Wound healing is a compound procedure that can be categorized into at minimum 3 phases: (1) an inflammation phase, (2) a proliferative phase resulting in tissue restoration, and (3) last phase including tissue remodeling. These processes are adjusted by numerous cytokines and growth factors which are liberated into the injury place (Li *et al*., 2007). The 1st stage includes cellular and vascular episodes and is described by erythema, edema, and marked local hyperemia. At the 2nd stage, epithelial tissue is formed accompanied with growth of granulation tissue and new vessels (angiogenesis). The process of angiogenesis looks to be sharply controlled by numerous cytokines and growth factors. Just the tissue inside the injury site is maintained, the maturation stage starts (Reinke and Sorg, 2012). The formation of extracellular matrix constituents and collagens elevates the stretchy power of the wound. The last result of this approach of curing is the formulation of tissue capable of substituting the damaged skin (Chodorowska and Roguś-Skorupska, 2004). In this study, the process of angiogenesis was marked depending on the beneficial effects of PP powder and BMPP medication.

Diabetic wound healing is delayed due to persistent inflammation, and macrophage-immunomodulating biomaterials can control the inflammatory phase and shorten the healing time (Zhang *et al*., 2024). Based on the findings of the later study, human-derived embryonic stem cell or induced pluripotent stem cell-derived acellular embryoid bodies can be used to promote diabetic wound healing in combination with hydroxybutyl chitosan hydrogels for clinical translation in the future (Zhang *et al*., 2024). Ding *et al*., (2024) have also reported that inorganic zinc mineralized diatom biosilica and hydroxybutyl chitosan composite hydrogel have the potential to be used as a therapeutic dressing for diabetic chronic wounds. Louiselle *et al*., (2021) concluded that treatment of diabetic wounds will likely require a multi-modal approach including management of underlying diabetes and control of hyperglycemia, topical therapeutics, and prevention of secondary infection and inflammation.

Concerning wound healing, this study showed that when using PPBM, the time required for healing was the shortest compared to the control group without any medication (C1). Additionally, the time required

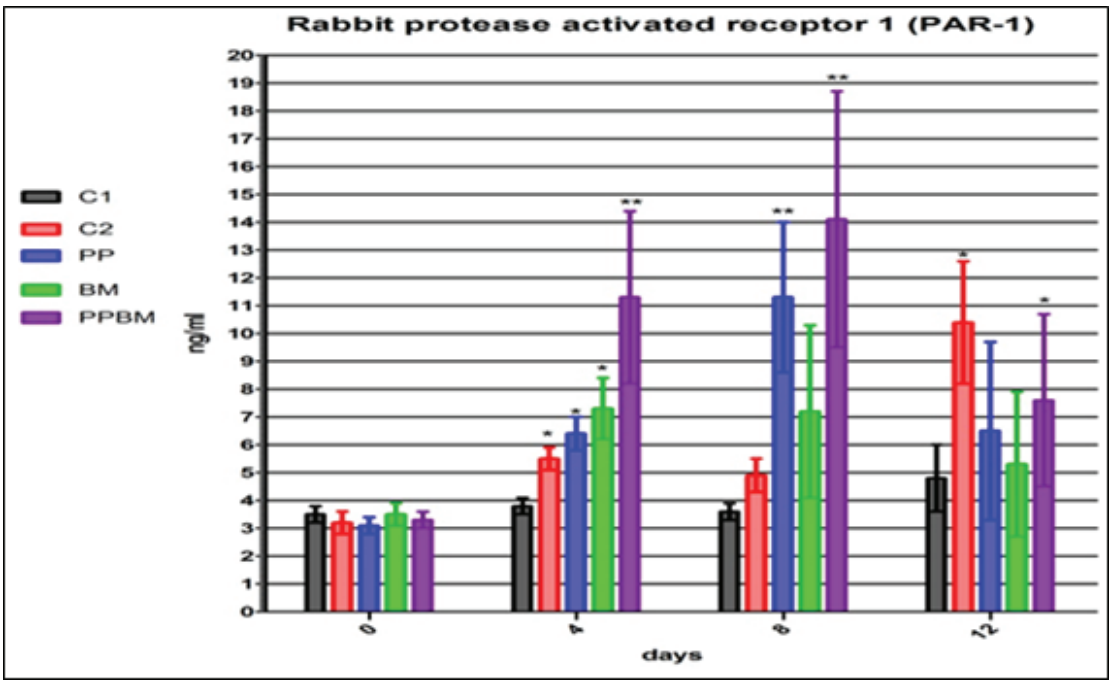


**Fig. 5.** Mean (±SE) level of concentration for rabbit protease-activated receptor 1 (RPAR-1) at days 0, 4, 8, and 12.

when using PP alone was shorter than that required when using BM alone. In another study, it was found that a powder of standardized pomegranate extract of 10% increases the healing process of deep second-degree burn wounds (Lukiswanto *et al*., 2019). It was concluded from a recent report that pomegranate seed oil improves wound-healing properties (Uzunhisarcıklı and Yerer, 2021). Furthermore, Yan *et al*., (2013), found that PP polyphenols gel may be a beneficial agent for curing wound disorders in alloxan-induced diabetic rats.

It was also found in this study that BM-derived mesenchymal stem cell transplantation exhibits promising therapeutic potential in diabetic foot ulcer treatment (Dama *et al*., 2023). Using pre-treated BM cells with sodium nitroprusside enhanced chronic injury curing with (Fatima *et al*., 2022). Direct insertion of BM-originated mesenchymal stem cells to the injured site resulted also in enhanced tissue repair through the release of paracrine factors (Wu *et al*., 2007). Human BM-mesenchymal stem cells transplantation was also found to facilitate development and improve burn injury and improve wound curing in a mouse model of burn wounds (Xue *et al*., 2013). Transplantation of BM cells pre-activated with sodium nitroprusside improved also acute wound curing in rabbits (Fatima *et al*., 2023). Parallel to previous reports, results of this study clearly show that using PP powder alone or with BM accelerate significantly the healing process of burn wounds in alloxan-induced diabetic rabbits.

Our results showed also that the histopathological assessment revealed a marked elevation in the fibroblast in PP and PPBM groups versus other treatments. This might explain the speed of wound closure in these two groups compared to the others. During the process of wound healing, the migration and proliferation of fibroblasts are very important for extracellular matrix secretion. Fibroblasts are very animated cells that have a vital role in the repair of damaged tissue and also in fibrosis but the mechanism is poorly understood (Talbott *et al*., 2022). It was reported also that fibroblasts are crucial in backing normal wound curing, included in key events as dissolution the fibrins, formation of collagen and new extra cellular matrix structures to back up the other cells linked with influential wound curing, as well as shrinking wounds (Bainbridge, 2013). Parallel to fibroblasts, our results showed that the histological assessment revealed a significant elevation in the collagen in PP and PPBM groups versus others; a factor why wound healing in PP and PPBM groups were faster than other groups. Collagen is the major part of extracellular matrix that contributes to wound strength (Li *et al*., 2007). Collagen is also considered as one of biomaterials that is considered to be the crucial component of most of the formulations being developed for wound healing (Chattopadhyay and Raines, 2014; Sharma *et al*., 2022).

Biomarkers are biological indicators than can assess the events, processes, and circumstances happening within the living organism. These markers can evaluate either physiological or pathophysiological events occurring due to a specific disease. In the veterinary field, the discovery of biomarkers is rapidly emerging with multiple potentials for development

and implementation. Focus on biomarkers research is relevant not only to the health and welfare of food production and companion animals, but also to larger themes, such as worldwide food security. During the past 12 years, our research group has focused intensively on the usage of these biomarkers for the evaluation of health status of domesticated animals, their welfare, application in the early recognition of several diseases and disorders and as a prognostication indicator (Tharwat *et al*., 2012; Tharwat, 2012; Tharwat *et al*., 2013a,b,c,d; Tharwat *et al*., 2014a,b; Tharwat and Al-Sobayil, 2014a,b,c; Tharwat and Al-Sobayil, 2015a,b; Tharwat and Al-Sobayil, 2018a,b; Tharwat and Al-Sobayil, 2020; Tharwat, 2020a,b,c,d,e; Tharwat and El-Deeb, 2021; Tharwat, 2021; Tharwat and Al-Sobayil, 2022a,b; Tharwat, 2023; Tharwat *et al*., 2024). It is trusted that progress in the field of biomarkers in the future will improve animal welfare results, decrease its suffering, and increase the economic impact of the stockholders (Perera *et al*., 2022).

Numerous cellular processes and mediators linked to wound curing are released during healing that can be used as biomarkers. Neutrophils, macrophages, platelets, and fibroblasts liberate cytokines and other molecules including interleukins, growth factors, and tumor necrosis factor-α, of which PDGF have the major significance (Patel *et al*., 2016). It was reported that clinical examination of wounds for these mediators could foretell which injury will cure and which will not, proposing the use of these mediators as biomarkers of wound curing. There is also evidence that the implementation of growth factors like PDGF will lessen the recovering process of non-healing and chronic wounds (Hahm *et al*., 2011; Patel *et al*., 2016). However, immunohistochemical staining such as CD31 and vimentin were not used to display angiogenesis and fibroblasts; this is a limitation of this study.

In this study, concerning the wound biomarker PDGF-AA and compared to baseline values, there was a significant elevation in all treatment groups (PP, BM, and BMPP). In general, treatment with growth factors is considered an effective method to speed wound healing (Okabe *et al*., 2013; Dinh *et al*., 2015). It has been reported that PDGF is a crucial controller of the deposition of extracellular matrix within the healing injuries (Pierce *et al*., 1991; Patel *et al*., 2016). The second wound biomarker PAR-1 also increased significantly in all treatment groups compared to baseline values until day 8 of treatment. However, values declined significantly at day 12 in all treatment groups compared to the control group. It has been reported that PARs under normal circumstances permit cells to react to the proteolytically changed micro-environment found during growth, inflammation, and wound curing (Hollenberg, 2002). Although, potential local toxicity is a treatment limitation, significant increases of the wound biomarkers PDGF-AA and PDGF-AA emphasizes that treatment promotes healing of diabetic wounds. This is specifically clarified when using a PPBM mixture that increases the formation of fibroblast and collagen.

## Conclusion

This study has showed that PPBM significantly enhanced a critical regulator of extracellular matrix deposition (the PDGF) which increased the fibroblast and collagen formation during burn wound healing. Therefore, it can be concluded that both PP powder combined with BM significantly accelerate the healing process of burn wounds in alloxan-induced diabetic rabbits.

## Acknowledgments

The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1).

***Conflict of interest***

The authors declare that there is no conflict of interest.

***Authors' Contributions***

Conceptualization and design: FA; Practical work: FA and MA; formal analysis and interpretation of data: FA, MT; writing-original draft preparation: FA and MT; All authors revised and approved the final manuscript for publication.

***Funding***

This research received no external funding.

***Data availability***

All data supporting the findings of this study are available within the manuscript and no additional data sources are required.

## References

Abdullahi, A., Amini-Nik, S. and Jeschke, M. G. 2014. Animal models in burn research. Cell. Mol. Life Sci. 71, 3241–3255.

Abramov, Y., Golden, B., Sullivan, M., Botros, S. M., Miller, J. J., Alshahrour, A., Goldberg, R. P. and Sand, P. K. 2007. Histologic characterization of vaginal *vs.* abdominal surgical wound healing in a rabbit model. Wound Repair Regen. 15, 80–86.

Bainbridge, P. 2013. Wound healing and the role of fibroblasts. J. Wound Care 22, 407–408.

Burgess, J. L., Wyant, W. A., Abdo Abujamra, B., Kirsner, R. S. and Jozic, I. 2021. Diabetic wound-healing science. Medicina 57, 1072.

Burgess, M., Valdera, F., Varon, D., Kankuri, E. and Nuutila, K. 2022. The immune and regenerative response to burn injury. Cells 11, 3073.

Chattopadhyay, S. and Raines, R. T. 2014. Review collagen-based biomaterials for wound healing. Biopolymers 101, 821–833.

Chodorowska, G. and Roguś-Skorupska, D. 2004. Cutaneous wound healing. Ann. Univ. Mariae Curie Sklodowska Med. 59, 403–407.

Dama, G., Du, J., Zhu, X., Liu, Y. and Lin, J. 2023. Bone marrow-derived mesenchymal stem cells: A

promising therapeutic option for the treatment of diabetic foot ulcers. Diabetes Res. Clin. Pract. 195, 110201.

Ding, Y., Mu, Y., Hu, Y., Liu, J., Su, C., Sun, X., Chen, X., Jia, N. and Feng, C. 2024. Zinc-mineralized diatom biosilica/hydroxybutyl chitosan composite hydrogel for diabetic chronic wound healing. J. Colloid Interface. Sci. 656, 1–14.

Dinh, T., Braunagel, S. and Rosenblum, B. I. 2015. Growth factors in wound healing: the present and the future? Clin. Podiatr. Med. Surg. 32, 109–19.

Dziewulski, P. 1992. Burn wound healing: James Ellsworth Laing Memorial Essay for 1991. Bums18, 466–478.

Fatima, N. and Saleem, M. 2023. Transplantation of bone marrow cells preactivated with sodium nitroprusside improves acute wound healing in rabbits. Int. J. Low. Extrem. Wounds. 22, 531–541.

Fatima, N., Saleem, M. and Shahbaz, U. 2022. Improvement of chronic wound healing by pre-activated bone marrow cells with sodium nitroprusside in rabbits. Drug Res.72, 139–147.

Greenhalgh, D. G. 2003. Wound healing and diabetes mellitus. Clin. Plast. Surg. 30, 37–45.

Hahm, G., Glaser, J. J. and Elster, E. A. 2011. Biomarkers to predict wound healing: the future of complex war wound management. Plast. Reconstr. Surg. 127 Suppl 1, 21S–26S.

Hollenberg, M. D. 2002. Compton SJ: International Union of Pharmacology. XXVIII. Proteinase-activated receptors. Pharmacol. Rev. 54, 203–217.

Li, J., Chen, J. and Kirsner, R. 2007. Pathophysiology of acute wound healing. Clin. Dermatol. 25, 9–18.

Louiselle, A.E., Niemiec, S.M., Zgheib, C. and Liechty KW. 2021. Macrophage polarization and diabetic wound healing. Transl. Res. 236, 109–116.

Lukiswanto, B. S., Miranti, A., Sudjarwo, S. A., Primarizky, H. and Yuniarti, W. M. 2019. Evaluation of wound healing potential of pomegranate (*Punica granatum*) whole fruit extract on skin burn wound in rats (*Rattus norvegicus*). J. Adv. Vet. Anim. Res. 6, 202–207.

Markiewicz-Gospodarek, A., Kozioł, M., Tobiasz, M., Baj, J., Radzikowska-Büchner, E. and Przekora, A. 2022. Burn wound healing: clinical complications, medical care, treatment, and dressing types: the current state of knowledge for clinical practice. Int. J. Environ. Res. Public Health. 19, 1338.

Murthy, K. N., Reddy, V. K., Veigas, J. M. and Murthy, U. D. 2004. Study on wound healing activity of *Punica granatum* peel. J. Med. Food. 7, 256–259.

Okabe, K., Hayashi, R., Aramaki-Hattori, N., Sakamoto, Y. and Kishi, K. 2013. Wound treatment using growth factors. Modern Plast. Surg. 3, 108–112.

Okonkwo, U.A. and DiPietro, L.A. 2017. Diabetes and Wound Angiogenesis. Int. J. Mol. Sci. 18, 1419.

Patel, S., Maheshwari, A. and Chandra, A. 2016. Biomarkers for wound healing and their evaluation. J. Wound Care. 25, 46–55.

Perera, T. R.W., Skerrett-Byrne, D. A., Gibb, Z., Nixon, B. and Swegen, A. 2022. The future of biomarkers in veterinary medicine: emerging approaches and associated challenges. Animals 12, 2194.

Pierce, G. F., Mustoe, T. A., Altrock, B. W., Deuel, T. F. and Thomason, A. 1991. Role of platelet-derived growth factor in wound healing. J. Cell Biochem. 45, 319–26.

Prompers, L., Schaper, N., Apelqvist, J., Edmonds, M., Jude, E., Mauricio, D., Uccioli, L., Urbancic, V., Bakker, K., Holstein, P., Jirkovska, A., Piaggesi, A., Ragnarson-Tennvall, G., Reike, H., Spraul, M., Van Acker, K., Van Baal, J., Van Merode, F., Ferreira, I. and Huijberts, M. 2008. Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. Diabetologia 51, 747–55.

Reinke, J. M. and Sorg, H. 2012. Wound repair and regeneration. Eur. Surg. Res. 49, 35–43.

Schlager, A., Kronberger, P., Petschke, F. and Ulmer, H. 2000a. Low-power laser light in the healing of burns: a comparison between two different wavelengths (635nm and 690 nm) and a placebo group. Laser Surg. Med. 27, 39–42.

Schlager, A., Oehler, K., Huebner, K. U., Schmuth, M. and Spoetl, L. 2000b. Healing of burns after treatment with 670-nanometer low-power laser light. Plast. Reconstr. Surg. 105, 1635–1639.

Sharma, S., Rai, V. K., Narang, R. K. and Markandeywar, T. S. 2022. Collagen-based formulations for wound healing: a literature review. Life Sci. 290, 120096.

Talbott, H. E., Mascharak, S., Griffin, M., Wan, D. C. and Longaker, M T. 2022. Wound healing, fibroblast heterogeneity, and fibrosis. Cell Stem Cell. 29, 1161–1180.

Tang, P. C., Rao, S. R. and Tang, Y. M. 2011. Advance of the extraction of pomegranate peel polyphenol and its activity. J. Xihua. Univ. (Nat. Sci. Edi.) 30, 98–102.

Tharwat M. 2020c. Inflammation and bone biomarkers in healthy dromedary camels with isoflurane or halothane general anesthesia. J. Camel Pract. Res. 27, 295–300.

Tharwat, M. 2012. The cardiac biomarker troponin I and other hematological and biochemical variables in downer camels (*Camelus dromedarius*). J. Camel Pract. Res. 19, 123–128.

Tharwat, M. 2020a. Serum concentration of bone metabolism biomarkers in goats during the transition period. Vet. Med. Intern. 2020, 4064209.

Tharwat, M. 2020b. Biomarkers of infection and inflammation in camels (*Camelus dromedarius*). J. Camel Pract. Res. 27, 159–163; doi: 10.5958/2277-8934.2020.00023.5

Tharwat, M. 2020d. The cardiac biomarkers troponin I and creatine kinase myocardial band in camels (*Camelus dromedaries*) – review. J. Camel Pract. Res. 27, 121–128.

Tharwat, M. 2020e. *Hyalomma dromedarii* ticks induce a distinct acute phase reaction in dromedary camels. J. Camel Pract. Res. 27, 329–332.

Tharwat, M. 2021. Influence of 8 km training on cardiac biomarkers alongside hematobiochemical profiles in race camels. J. Camel Pract. Res. 28, 79–84.

Tharwat, M. 2023. Advanced biomarkers and its usage in Arabian camel medicine – a review. J. App. Anim. Res. 51, 350–357.

Tharwat, M. and Al-Sobayil, F. 2014a. Influence of transportation on the serum concentrations of the cardiac biomarkers troponin I and creatine kinase myocardial band (CK-MB), and on cortisol and lactate in horses. J. Equine Vet. Sci. 34, 662–667.

Tharwat, M. and Al-Sobayil, F. 2020. A review on biomarkers of bone metabolism in camels (*Camelus dromedaries*). J. Camel Pract. Res. 27, 23–29.

Tharwat, M. and El-Deeb, W. 2021. Biomarkers of stress in healthy and diseased dromedary camels. J. Camel Pract. Res. 28, 297–302.

Tharwat, M., Ali, A., Al-Sobayil, F., Derar, R. and Al-Hawas, A. 2014b. Influence of stimulation by electroejaculation on myocardial function, acid-base and electrolyte status and haematobiochemical profiles in male dromedary camels. Theriogenology 82, 800–806.

Tharwat, M., Ali, A., Derar, D., Oikawa, S. and Almundarij, T. I. 2024. Effects of dystocia on the cardiac biomarker troponin I, acid-base balance and blood gases alongside the hematobiochemical profiles in female dromedary camels. Int. J. Vet. Sci. 13, 115–119.

Tharwat, M., and Al-Sobayil, F. 2014b. The effect of tick infestation on the serum concentrations of the cardiac biomarker troponin I, acid–base balance and haematobiochemical profiles in camels (*Camelus dromedarius*). Trop. Anim. Health Prod. 46, 139–144.

Tharwat, M., and Al-Sobayil, F. 2014c. Influence of the cardiac glycoside digoxin on cardiac troponin I, acid–base and electrolyte balance, and haematobiochemical profiles in healthy donkeys (*Equus asinus*). BVC Vet. Res. **10,** 64.

Tharwat, M., and Al-Sobayil, F. 2015a. The impact of racing on serum concentrations of acute-phase proteins in racing dromedary camels. Comp. Clin. Pathol. 24, 575–579.

Tharwat, M., and Al-Sobayil, F. 2015b. Serum concentrations of acute phase proteins and bone biomarkers in female dromedary camels during the periparturient period. Journal of J. Camel Pract. Res. 22, 271–278.

Tharwat, M., and Al-Sobayil, F. 2018a. Influence of electro-ejaculator on serum concentrations of acute phase proteins and bone metabolism biomarkers in male dromedary camels (*Camelus dromedarius*). J. App. Anim. Res. 46, 1226–1232.

Tharwat, M., and Al-Sobayil, F. 2018b. The impact of racing on serum concentrations of bone metabolism biomarkers in racing Arabian camels. J. Camel Pract. Res. 25, 59–63.

Tharwat, M., and Al-Sobayil, F. 2022a. The Effects of acute blood loss on inflammatory and bone biomarkers, acid base balance, blood gases and hemato-biochemical profiles in sedated donkeys (*Equus asinus*). Int. J. Vet. Sci. 11, 479–485.

Tharwat, M., and Al-Sobayil, F. 2022b. Effect of a long road transport journey on serum biomarkers of bone formation and resorption in athletic horses. Int. J. Vet. Sci. 11, 268–271.

Tharwat, M., Al-Sobayil, F. and Ahmed, A. F. 2013c. Effect of isoflurane and halothane on myocardial function in healthy dromedary camels as assessed by cardiac troponin I. J. Camel Pract. Res. 20, 289–294.

Tharwat, M., Al-Sobayil, F. and Al-Sobayil, K. 2012. The cardiac biomarkers troponin I and CK-MB in nonpregnant and pregnant goats, goats with normal birth, goats with prolonged birth, and goats with pregnancy toxemia. Theriogenology 78, 1500–1507.

Tharwat, M., Al-Sobayil, F. and Buczinski, S. 2013a. Influence of racing on the serum concentrations of the cardiac biomarkers troponin I and creatine kinase myocardial band (CK-MB) in racing greyhounds. Vet. J. 197, 900–902.

Tharwat, M., Al-Sobayil, F. and Buczinski, S. 2013d. Cardiac biomarkers changes in camels (*Camelus dromedarius*) secondary to long road transportation. J. Vet. Cardiol. 15, 15–22.

Tharwat, M., Al-Sobayil, F. and Buczinski, S. 2014a. Influence of racing on the serum concentrations of acute phase proteins and bone metabolism biomarkers in racing greyhounds. Vet. J. 202, 372–377.

Tharwat, M., Al-Sobayil, F. and El-Sayed, M. 2013b. Cardiac troponin I in healthy newborn goat kids and in goat kids with cardiac nutritional muscular dystrophy. Acta Vet. Hung. 61, 442–453.

Uzunhisarcıklı, E. and Yerer, M. B. 2021. Role of Hypericum perforatum oil and pomegranate seed oil in wound healing: an *in vitro* study. Z. Naturforsch. C. J. Biosci. 77, 189–195.

Wang, J., Wan, R., Mo, Y., Zhang, Q., Sherwood, L. C. and Chien, S. 2010. Creating a Long-TermDiabetic RabbitModel. Exp. Diabetes Res. 2010, 1–10.

Wu, Y., Wang, J., Scott, P. G. and Tredget, E. E. 2007. Bone marrow-derived stem cells in wound healing: a review. Wound Repair Regen. 15 Suppl 1, S18–26.

Xue, L., Xu, Y. B., Xie, J. L., Tang, J. M., Shu, B., Chen, L., Qi, S. H. and Liu, X. S. 2013. Effects of

human bone marrow mesenchymal stem cells on burn injury healing in a mouse model. Int. J. Clin. Exp. Pathol. 6, 1327–1336.

Yan, H., Peng, K. J., Wang, Q. L., Gu, Z. Y., Lu, Y. Q., Zhao, J., Xu, F., Liu, Y. L., Tang, Y., Deng, F. M., Zhou, P., Jin, J. G. and Wang, X. C. 2013. Effect of pomegranate peel polyphenol gel on cutaneous wound healing in alloxan-induced diabetic rats. Chin. Med. J. 126, 1700–1706.

Zhang, Y., Chen, Z.H., Zhao, K., Mu, Y.D., Li, K.L., Yuan, Z.M., Liu, Z.G., Han, L., and Lü, W.D. 2024. Acellular embryoid body and hydroxybutyl chitosan composite hydrogels promote M2 macrophage polarization and accelerate diabetic cutaneous wound healing. Mater Today Bio. 25, 100975.