The Effects of a Novel Caprine Acellular Dermal Powder Hydrogel on The Healing of Full-Thickness Cutaneous Wounds in Iraqi Bucks

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ABSTRACT
Skin wounds are important problems which causing suffering for animals and their owners, and their treatment is necessary for animal health, so the purpose of this study is to evaluate how well caprine acellular dermal powder hydrogel (GADPH) promotes the healing of cutaneous wounds in Iraqi bucks. 48 of full-thickness cutaneous wounds (3X3 cm) created on the dorsal back of twelve bucks, each two wounds on both sides. Then, divided into two groups according to treatment method (6 bucks /group). The wounds in the control group (A), were left without treatment. The wounds in treatment group (B) were treated by applying GADPH to the wound beds. The outcomes were assessed clinically (scoring) at 7, 14, and 35 days post treatment (4 samples/animal), and gene expression by RT-PCR of two genes including fibroblast growth factor (FGF) and epidermal growth factor (EGF) at three times periods 0,7 and 14 days (5 samples/group) and histopathological assessment at (7,14, 35 and 42) days post treatment. Scoring results of treated wounds showed that the percentages of re-epithelization, contraction, and total wound healing were significantly higher than that of control wounds at the level of ($P<0.001$) from the first seven days until the end of the study. Also, gene expression of FGF and EGF was considerably higher in the treated group than the control group at the level of ($p<0.001$). The histopathological results in group B were showed faster re-epithelialization of the epidermis, proliferation of fibrin and regenerated epithelia, dense collagen fibers, and finally complete regeneration of the epidermal layer at the end of study as compared to group A which was showed slowly development in wound healing proses. In conclusion, GADPH enhanced and accelerated the healing of skin wounds in male goats.

1. Introduction

The skin is the largest organ tissue of the body, normally forming for 15-20% of total body weight and presenting 1.5-2 m^2 of surface to the outer environment (Yousef *et al*., 2022; Mahmood & Mahdi, 2022). Skin wound is described as the destruction or disruption of the typical anatomical structure and skin function (Khalaf & Salih, 2018). Skin injuries represent a major healthcare problem, complete loss of skin injury may result in extensive destruction to the skin and underlying tissues. According to Ghaima (2013) and Atiyah and Al-Falahi (2021), wound healing is a pathophysiology event that begins with the damage of skin integrity and results in a breaking of continuity that extends the beneath layers in varying stages. This process is dependent on a sequence of chemical responses that are traditionally categorized into three phases: inflammation, proliferation, and tissue remodeling (Ghaima, 2013(. Some wounds do not heal by themselves, and these require a skin substitute (Tavakoli and Klar, 2021). Continuing research goings-on to find new biomaterials which can be utilized, there are a range of treatment approaches appropriate including wound bandages (Carter *et al*., 2010), debridement (Dumville *et al*., 2009), and skin alternatives (Greaves *et al*., 2013).

Recently, the using of bioscaffold resources for different requests has enlarged dramatically over the last years (Turner & Badylak 2014). There are three main categories of materials involved in a skin substitute that must be considered during manufacturing: scaffolds, growth factors, and cells (Eldeeb *et al*., 2022). Biological scaffolds resulting from decellularized organs and tissues, such as porcine small intestinal sub mucosa, and a cellular dermal matrix, are being developed by surgeons to replace synthetic non absorbable materials, which may cause allergic reactions (AL-Bayati *et al*., 2016; Mahdi and AL-Bayati, 2019). Caprine acellular dermal matrix (ADM) is high in collagen mainly Type-I and III, also glycosaminoglycans (GAGs) and growth factors, glycoproteins, chemokines, and cytokines (Athar *et al*., 2014; Swinehart & Badylak, 2016). Hydrogels are composed of naturally occurring

biomaterials, including collagen, hyaluronic acid, fibrin alginate, and extracellular matrix proteins. These materials are suitable for use in the biomedical field because of their high-water content, good biocompatibility, ability to interact with living tissues, and elastic consistency (Claudio-Rizo *et al*., 2017). Because tissue-derived hydrogels retain the extracellular matrix's composition as well as the natural biological and chemical signals associated with these matrix constituents, they more closely resemble the original tissue (DeQuach *et al*., 2012; Omar and Serwa, 2019). So, the current experiment was planned to assess the GADPH hydrogel properties on curing full-thickness skin injuries.

2. Material and Methods Experimental Animals:

The current study was conducted in the animal house of the College of Veterinary Medicine /University of Baghdad, throughout the duration of the study (3) months. Twelve healthy mature local breed bucks weighing (15-20) kg and (1.5-2) years old were recruited for this study. The animals were kept in farm animal home of the Veterinary Medicine College/University of Baghdad. Clinical evaluation by a physical examination before initiation of the experiments were done to all animals. The animals were left for four weeks to adapt to the experimental condition. The animals received free access to water and food during the period of the experiment.

Ethics Statement

The trial work followed the approvals of the experimental animal care and the official Committee of University of Baghdad/college of veterinary medicine (number 1163 on 2024/0609). Every attempt was made to reduce the pain and distress of the experimental animals

Surgical Operation

Four Full-thickness cutaneous square wounds (3X3) centimeters were created on the dorsal back of 12 male goats (2 wounds on each side one cranial and one caudal), with 10 cm between each wound (**AL-Bayati *et al*., 2016)** (24 wounds/group) (**figure 1**). To induce full thickness cutaneous wounds on the dorsal area of each animal, all the animals were injected intramuscularly (I.M) with 10,000 IU and 10mg/kg body weight of (penicillin-streptomycin/Kepro-Holand)12 hours before surgery respectively, and submitted to surgical operation under sedation by IM injection 2% Xylazine hydrochloride at a dose of (0.2 mg/kg) and local anesthesia using inverted L local infiltration of lidocaine hydrochloride 2% at dose (10mg/kg B.W) in wound borders (**Sarkar *et al*., 2016)** then the animals were randomly split into two groups based on treatment method as follow:

A- Control group (n=6): The wounds were left without treatment only dressed using sterile gauze.

B- Treatment Group (n=6): The wounds were treated daily with local application of (GADPH) in the wound bed which is applied and dressed using sterile gauze.

Post-operative care

All wounds were covered with sterile gauze fixed with medical tape and changed every day (Elbialy *et al*., 2020), animals were injected with penicillin/streptomycin (10mg/kg IM) post-wounding for five days.

Clinical Evaluation

Full clinical examination was done daily on each animal throughout the weeks of study, then the wounds areas were shaved carefully, and digital photograph was taken on the day where it was made and then once every week. The scab wound was gently eliminated by using saline, to improve vision of areas of epithelialization and granulation tissue. The percentage of epithelialization, contraction and total healing of wound were estimated for each injury, based on the method stated by (Bohling *et al*., 2004), as the following:

1. Percent of epithelization: was estimated by % Epithelization (day n) =

[Area of epithelium (day n) / Total wound area (day 0)] x 100

2. Percent of wound contraction, computed by:

Step 1: Full wound size on (day n) as % of the original wound =

[Total wound area (day n) / Original wound area (day 0)] x 100

Step 2: % Wound contraction (day n) =

100 – Total wound size on (day n) as % of the original wound

3. Percent of total wound healing, measured by:

Step 1: Open wound size day n as % of the original =

[Open wound area (day n) / Original wound area (day 0)] x 100

Step 2: % Total wound healing (day n) = 100 – Open wound size (day n) as % of the original wound.

Molecular Study:

Gene expression of FGF & EGF genes by rt-PCR at **(0, 7 and 14)** days post wounding using primers in (**Table 6**). The primers were used in quantification of gene expression by using RT-qPCR techniques based BRYT Green DNA binding dye (Promega, USA).

RNA Purification:

RNA was separated from the sample depending on the protocol of TRIzol™ Reagent as the following steps:

A-Sample lysis

Tissues: For every tube, 0.5mL from TRIzol™ Reagent was added per 50-100 mg of sample and lightly mixed by the vortex.

B-For three-phase separations

•For all tube, 0.2 mL of chloroform was added to the lysate, then the tube cap was locked.

• All mixes were Incubated for 2–3 minutes and then centrifuged for 10 minutes at 12,000 rpm, the mixture was separated into a lower organic phase, interphase, and a colorless upper aqueous phase.

• The aqueous phase involving the RNA was transmitted to a new tube. **C-For RNA precipitation**

• 0.5 mL of isopropanol was added to the aqueous phase and incubated for 10 minutes then centrifuged for 2 minutes at 13000 rpm.

• Total RNA was precipitated and formed a white gel-like pellet at the lower of the tube.

• Supernatant was then disposed of. **D-For RNA washing**

• For each tube, 0.5mL of 70% ethanol was added and vortex briefly then centrifuged for 2 minutes at 13000 rpm.

• Ethanol then aspirated and air-dried the pellet. **E-For RNA solubility**

• Pellet was rehydrated in 50 µl of Nuclease Free Water then incubated in a heat block set at 55–60°C for 10–15 minutes.

Quantitation of RNA (Determination of RNA Concentration):

To detect the concentration of extracted RNA, Quantus Fluorometer was used to detect the quality of samples for downstream applications. 200µl of diluted QuantiFlour Dye was mixed with1 µl of RNA. After 5min incubation at room temperature in a dark place, RNA concentration values were detected.

Reaction Setup and Thermal Cycling Protocol:

As stated in table, (3.3), each step of RT-PCR for each gene of the current study contained the following data.

Table 1: Shows one-step RT-PCR.

PCR Component Calculation				
No. of Reaction	**45**	rxn	Annealing temperature of primers	**58,65**
Reaction Volume /run	**10**	µl	No. of primers	**3**

Quantitative Real-Time PCR (qPCR) Master Mix Preparation:

The one step RT-qPCR kit that is dependent BRYT Green dye discovering of gene amplification in the Real-Time PCR system was used to create the quantitative PCR (qPCR) master mix. The kit contents are listed in table, (3.4). next, utilizing the reaction technique mentioned in table, (3.5), the smart PCR tubes were placed in the smart cycler device.

Table (2): Shows the quantitative Real-Time PCR (qPCR) master mix Kit.

Master mix components	**Stock**	**Unit**	**Final**	**Unit**	**Volume**
					1 Sample
qPCR Master Mix	2	X	1	X	5
RT mix	50	x	1	x	0.25
MgCl2					0.25
Forward primer	10	µM	0.5	µM	0.5
Reverse primer	10	µM	0.5	µM	0.5
Nuclease Free Water					2.5
RNA		ng/µl		ng/µl	1
Total volume					10
Aliquot per single rxn	9µl of Master mix per tube and add 1µl of Template				

Preparation of Acellular Dermal Matrix (ADM):

The decellularization of ADM was performed as described by (Shevchenko *et al*., 2010) method with some changes. In summary, the skin of caprine origin was obtained from (Alshu'la slaughterhouse/Baghdad) and directly kept in ice cold sterile phosphate-buffered saline (PBS, pH 7.4) having proteolytic inhibitor (0.02% EDTA) and broad-spectrum antibiotic (Amikacin-1 mg/ml). Then completely the skin cleaned with sterile PBS to get rid of all the attached blood and remains. De-

epithelialization of the skin was accomplished by using 0.25% trypsin and 2 M sodium chloride solution for 8 hrs. After de-epithelization, the dermis was decellularized using 2% sodium deoxycholate for 72 hrs. with continual agitation of sample in a horizontal orbital shaker at the speed of 180 rotations/minute during the de-epithelization and decellularization procedure to deliver better interaction of tissue with chemicals. To evaluate the acellularity of the prepared matrix, a light microscope was used showed after staining by Hematoxylin and Eosin (H and E) staining of the representative samples. Finally, to eliminate the remaining chemicals ADM was washed 6 times (2 hours each) with sterile PBS and kept in PBS solution containing 0.1% amikacin solution at −20 °C. The frozen decellularized skin was thawed at room temperature, then sliced into pieces of **3 × 3** cm^2 and freezer-dried (lyophilized) for 24 h. The dehydrated pieces were ground in a cryo-mill with a 0.75-µm sieve at 14,000 rpm. The finished filamentous powder was stored in (4C˚) till use (Pridgen *et al*., 2011; Vijay *et al*., 2017).

Preparation of Hydrogel:

According to **Cai *et al* (2021)**, the prepared powder was enzymatically dissolved by adding of 1mg/ml pepsins solution with 0.02M (HCl) and distilled water, the concentration of prepared powder was 50 mg/ml and pH (2.2-2.4) following that continuous stirring on a magnetic stirrer at room temperature for 24 hrs. with. The resulting liquid was allowed to cool on ice while deactivate the pepsin enzyme by using (NaOH), raising the pH to 8 to and later the pH was dropped to (7.0-7.4), the salt content was regulated by using 10x PBS to access an isotonic solution, then the hydrogel kept at 4ºC in the refrigerator (**figure 2**).

Histopathological Evaluations:

The histological evaluation was performed on days (7, 14, 28, and 35) post-wounding in both control and treatment groups (six wounds/period). Full-thickness of (5-6) mm biopsy samples were taken, and they involved about (3-4) mm of normal skin on both sides of the wound which were fixed in (10%) neutral formalin solution, and then inserted in paraffin and then sectioned in (5- 7) micron on a rotary microtome and finally stained with Hematoxylin-Eosin (H&E) (Luna, 1992).

Statistical Analysis:

The mean and standard errors of sustained variables were stated, and significant differences were tested using the analysis of variance (ANOVA) test for days or independent samples T-test for groups, followed by the least significant difference (LSD) test. Statistical significance was defined as a probability value ($p \leq 0.05$) and ($p \leq 0.001$) (Snedecor & Cochran**,**1973).

Fig. 1: Show's, the shape, and sites of four injuries on lateral thoracic areas.

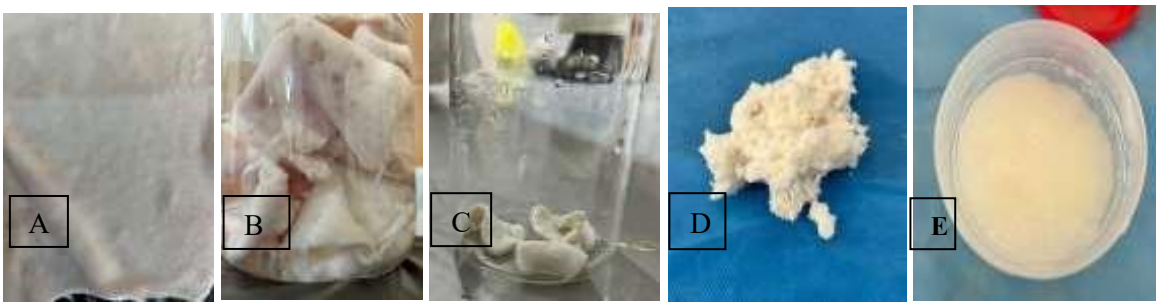

Fig. 2: Show's steps of GADPH preparation, (A) Goat skin after shaving, (B) ADM, (C) 3X3 cm pieces (D) lyophilized powder (E) prepared hydrogel.

3. Results and Discussions:

Clinical findings:

Neither group experienced any problems such as wound infection or extensive bleeding during the periods of research. Within the first 24 hours after wounding, the animals had normal eating, urination, and defecation until the end of study. This might be attributed to the use of extremely effective antibacterial and suitable post-operative care, these results were consistent with Elce *et al*., (2005) who used GADM for treatment of abdominal wall hernia, no problems were detected after retroperitoneal using of ADM in any animals. These findings also were agreed with Azari *et al*., (2008), in goat wounds which healed by second intention without any complications. Such findings in Albayati *et al*., (2013) study, no problems such as infection of wound or vigorous granulation tissue formation in the wound sites. Same results seen by Kumar *et al.* (2013) no post-surgery complications seen following retroperitoneal placement of goat ADM in any animals at minimum to 6 months after their repair.

A. Percentage of Epithelialization:

The epithelialization development in treatment wounds has a significant difference ($p \leq 0.001$) than that of control group, initiated from 14 days post wounding which was (25.91±1.45) in control group and (40.50±3.97) in treatment group. Then, the percentage of epithelialization in control group was (83.50±0.80) while in treatment group was (97.83±1.60) at day 35 (see Table 3).

Table 3: Show's the means and standard error of re-epithelialization percentage in control and treatment groups

Periods Groups	7 days Mean ±SE.	14 days Mean ±SE.	28 days Mean ±SE.	35 days Mean ±SE.
Control A group	14.25±1.63	25.91±1.45	63.00±2.80	83.50±0.80
group B Treated	16.17±0.74	40.50±3.97	75.00±3.23	97.83±1.60
p-value LSD	0.000* 6.38			

*High significant differences at probability value (p≤0.001).

B. Percentage of Wound Contraction:

Speed of wound contraction was significant differences (p≤0.001) between control and treatment groups started from **7** days post wounding, it was **(20.00±1.84)** in control group and **(25.67±1.67)** in treatment group**,** then at **35** day of the study reach (**88.75±1.29)** in control group and **(99.17±0.65)** in treatment group (see **Table 4**).

Table 4: Show's the means and standard error of wound contraction percentage in control and treatment groups

Periods Groups	7 days Mean ±SE.	14 days Mean ±SE.	28 days Mean ±SE.	35 days Mean ±SE.
Group A control	20.00±1.84	35.67±2.01	71.67±1.87	88.75±1.29
Group B treatment	25.67±1.67	46.50±1.23	83.42±1.55	99.17±0.65
p-value LSD	0.000* 4.03			

*High significant differences at probability value (p≤0.001). **C.**

Wound healing:

The wound healing ratio showed the significant differences (p≤0.001) amongst two groups began from **7** days post-injury, it was (**26.17±1.62**) in control group and (**A46.00±1.29)** in treatment group, then reach (**89.83±1.35)** in the control group and **(100.00±0.00)** at **35** days (see **Table 5**).

Table 5: Show's the means and standard error of wound Recovery percentage in control and treatment groups.

Periods Groups	7 days Mean ±SE.	14 days Mean ±SE.	28 days Mean ±SE	35 days Mean ±SE.
Group A control	**26.17±1.62**	**40.33±1.71**	**78.00±0.73**	**89.83±1.35**
Group B treated	**46.00±1.29**	**72.67±3.35**	**95.83±1.70**	**100.00±0.00**
p-value	0.000*			
LSD	4.48			

*High significant differences at probability value (p≤0.001).

The morphological calculation of wounds in this study throughout the course of the four-periods trial exhibited that, the epithelium in the wounds treated with GADPH progressed more quickly than in the control wounds. These results may be due to the properties of GADPH which preserve the natural components of goat skin and ECM which contain growth factors such as (bFGF, PDGF, TGF and EGF) and collagens, these factors facilitate epithelialization and improve dermal and epidermal renewal (Clarke *et al*., 1996; Mohammed and Salih, 2022). these findings agree with Cornwell *et al.,* (2009) who mention that dermal replacement scaffolds speed and improve dermal and epidermal regeneration by encouraging fibroblast adhesion, proliferation, and infiltration. Re-epithelialization begins within hours following damage, and the release of FGF, TGF-a, and EGF promotes the migration and proliferation of epithelial cells. Also, Barrientos *et al*., (2008), mention that cell-to-cell and cell-substratum connections break down at the start of this process, which is then followed by keratinocyte polarization and migration over the temporary extracellular matrix. Same results found in Kuna *et al*., (2017) study, the ECM gel supplying excellent adherence of cells and a moist healing situation, so that the gel reacted efficiently and protected the wound in the nude mouse model

The results of existing study demonstrated that wound contraction percentages were higher in treatment wound compared to the control wound, contraction and epithelialization are the processes that lead to closure in second intention wound healing (Singh *et al*., 2017). Similar results mentioned by Al-Bayati *et al*., (2013) which reported that wound contraction happens more quickly than epithelialization, it contributes more to rapid healing. The centripetal movement of the initial wound edges is known as wound contraction. The contraction of myofibroblasts in granulation tissue is the cause of this process (Swaim *et al.* 2001). Myofibroblasts are necessary for the contraction and repair of wounds. Their characteristics include the existence of tension fibers with the α-actin isoform, which is expressed in smooth muscle, and their differentiation from fibroblast. The activation of fibroblast and differentiation into myofibroblast is by (TGF) and increasing the number of cells to stimulate wound contraction (Merket *et al*., 2021).

Table 6: showing primers which are used in rt-PCR.

Primer Name	Sequence 5`-3`	Annealing Temp. (ºC)
EGF	F-(TCCCAGGTTCTCTTAAGTGCCT) R- (AACAGCCGCTTATCAAGCACATCC) **(Frota, *et al.*, 2010)**	58
FGF2	F-(AGTGTGTGCAAACCGTTACCTTGC) R- (ATACTGCCCAGTTCGTTTCAGTGC) **(Almeida, *et al.*, 2012)**	65
GAPDH	F-(TGTTTGTGATGGGCGTGAACCA) R-(ATGGCGTGGACAGTGGTCATAA) **(Almeida, *et al.*, 2012; Kate, 2019)**	65

Fig. 3: Shows wounds healing in control (C) and treatment (T) groups at different periods of study.

Molecular Outcomes:

Results of the genes levels in the current study revealed that existence of notable differences in the mean quantities of FGF &EGF genes within treatment and control groups at different follow-up periods. It was evident that there were high mean values of these genes at **7** and**14** days post wounding in the animals of treatment group than control group. The mean values of FGF gene were (**3.15±0.29**) in control group and (**5.71±0.03**) in treated group at **7** days post wounding while in 14 days was (**4.12±0.05**) in control and (**6.23±0.23**) in treated group (see **Table 7**). The results of EGF gene also were showed significant differences (p>0.001) in treated group higher than control group, it was (**2.57±0.03**) in control group, and (**4.31±0.10**) in treated group at **7** days post wounding. Then **14** days post wounding the level of gene expression were elevated in both groups with significant differences, it was (**2.99±0.00**) in control group and (**6.84±0.09)** in treated group (see **Table 8**).

Table (7): shows the mean values of FGF gene in RT-PCR. In control and treatment groups.

Periods Groups	0 day Mean ±SE.	7 days Mean ±SE.	14 days Mean ±SE.
Group A control	0.81±0.10	3.15±0.29	4.12±0.05
Group B treated	0.99±0.06	5.71±0.03	6.23±0.23
p-value	0.001*		
LSD	0.28		

*Significant differences at probability value (p≤0.05).

Table (8): shows the mean values of EGF gene in RT-PCR. In control and treatment groups.

Periods Groups	0 day Mean ±SE.	7 days Mean ±SE.	14 days Mean ±SE.
Group AC	0.79±0.01	2.57±0.03	2.99±0.00
Group BG	0.92±0.02	4.31±0.10	6.84±0.09
p-value	0.05**		
LSD	0.13		

**Significant differences at probability value (p≤0.05).

The current study appeared that the increasing in expression of FGF and EGF genes in wound site tissues, in group (**B)** at **7** days post-treatment showed the superiority than that in wound site of group (**A**), due to linked to the inflammatory response and recovery advance, characterized by highly inflammatory cell infiltration as a result release of b-FGF and EGF, which occur due to the effects of hydrogel on the host tissue and inflammatory cell-released b-FGF and EGF **(Al-Ebadi, 2018; Kate, 2019)**. This process was explained by **Badylak and Gilbert, (2008)**, who states that every biologic substance stimulates an acute host response, which is represented by a strong MNC invasion.

Londono and Badylack, (2015), referred that biomaterials can induce a transition from an inflammatory process to a constructive remodeling and functional tissue restoration process, hence modulating the various phases of the healing response. Other studies indicated that the composition of the biomaterial may influence the level and profile of the inflammatory reaction (Wooley *et al*., 2002). The elevation in b-FGF gene expression in B group indicating more proliferation of fibroblasts that lead to increase of collagen deposition (Areeg and Ahmed 2022). According to Badylack *et al.* (2008) research which declares that the acute host response is regular to all biologic scaffolds, which distinguished by an sever MNCs infiltration. The b-FGF is increased in the acute wound because it plays a role in granulation tissue formation, re-epithelialization, and tissue remodeling Barrientos *et al*. (2008). The early angiogenesis and repair of surgical wounds are partially mediated by b-FGF, which

can be released by cellular injury selectively (Al-Ebadi 2018). The use of biological materials in wound healing increases the level of b-FGF at the wound site due to their ability to attract inflammatory cells, which secrete growth factors, which leading to gradual increasing in levels started from 5 to 30 days after operation (Hammoodi, 2019). According to another research reported that surgical wounds heal quickly wounds, this mediated by b-FGF, which can be selectively released by cellular injury (Gonzalez *et al*., 2016; Mahdi, 2018).

In current study the level of EGF genes increased in all groups at 7 and 14 days of treatment, this may be due to remains of re-epithelialization process by promoting the migration and proliferation of epithelial cell which ends with complete healing. The main role of EGF in acute injuries is reepithelialization by promoting keratinocytes which consider the key cell components of the epithelium (Barrientos *et al*., 2008). Shiraha *et al*., (1999) mention that in acute wounds, EGF is principally produced by platelets, macrophages and fibroblasts and is regulated within a short interval of time following injury. EGF gene, released through cellular responses in wound sites, stimulates reepithelialization and is upregulated during early healing days when growth is maximal. It aids in reepithelialization by promoting the migration and proliferation of epithelial cells (Haase *et al*., 2003). H&E findings demonstrate that good bioactive properties in treated group by GADPH increasing the expression of b-FGF and EGF which are helpful for skin wound healing, same results showed in (Mohammed & Salih, 2022) study, the caprine ADM delivers perfect environment for renewal of the wound.

Histopathologic findings

The histopathological sections of skin biopsies were taken from control group at 7 days showed hemorrhage, necrotic tissue, and neutrophils infiltration, (figure 4) while treated group revealed proliferation of fibrin and inflammatory cells infiltration mainly neutrophils and MNCs., proliferation of fibrous tissue in the dermis (figure 5). At 14 days control group was showed focal aggregation of inflammatory cells consist of PMNCs and MNCs surrounded by irregular collagenous tissue (figure 6), while treated groups shows regenerated epithelia, whereas the dermis showed proliferation of fibrous tissue, present of fibroblast and irregular collagen bundles (figure 7). At 28 days post wounding control group shows incomplete regenerated epithelia with cellular debris (figure 8), while treated group show regenerated epithelia of the dermis revealed finger like projection deep in the dermal layer with proliferation of fibroblasts and fibrocytes (figure 9). Finally at 35 days post wounding control group were showed dense fibrous tissue and thick regenerated epithelia with hyperactive basal layer (figure 10), while treated group were showed complete regeneration of the epidermal layer and dense fibrous tissue seen in the dermal layer with many hair follicles (figure 11).

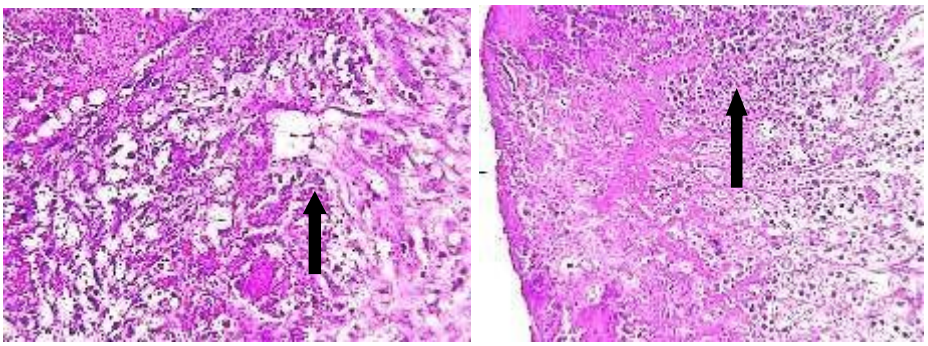

Fig 4: Histopathological section of skin wound from goat in control group at **7** days post wounding, shows hemorrhage, necrotic tissue, and neutrophils infiltration (black arrows) (H & E stain 400x, 200x).

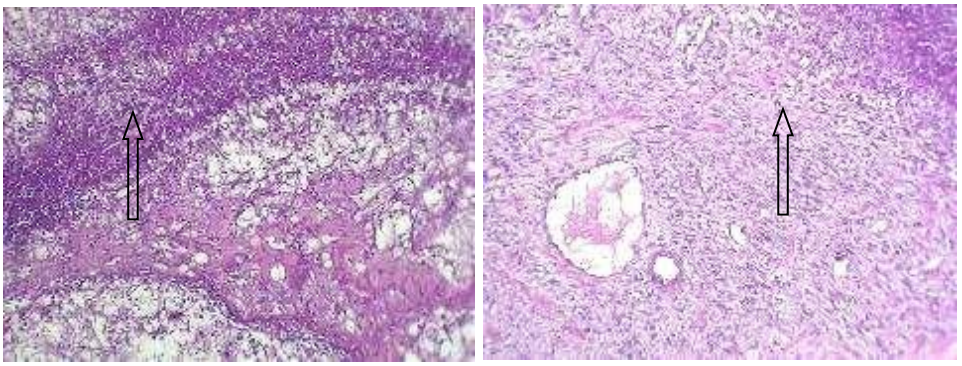

Figure 5: Histopathological section of skin wound from goat in treated group at **7** days post wounding, proliferation of fibrin and infiltration of inflammatory cells mainly neutrophils and MNCs (white arrows) (H & E stain 200×).

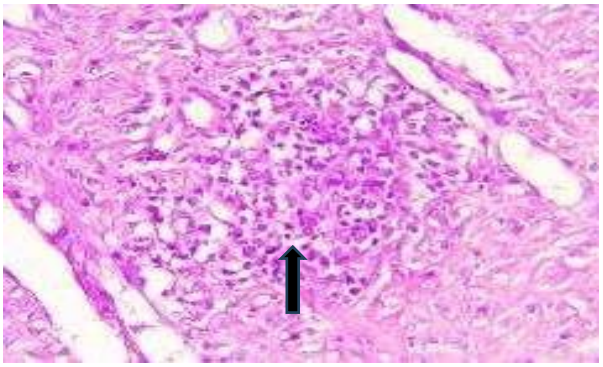

Fig. 6: Histopathological section of skin wound from goat in control group at **14** days post wounding, shows focal aggregation of inflammatory cells consisting of PMNCs and MNCs surrounded by irregular collagenous fiber (black arrow) (H & E stain 100×).

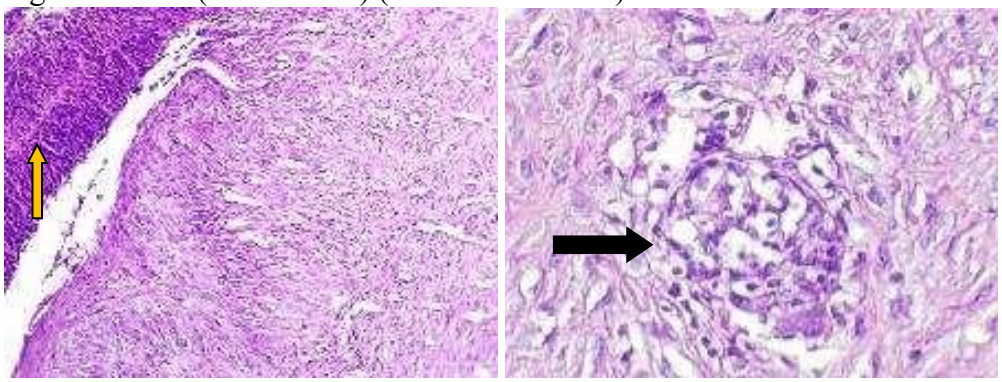

Figure 7: Histopathological section of skin wound from goat in treated group at **14** days post wounding regenerated epithelia (yellow arrow), while the dermis showed proliferation of fibrous tissue, with focal aggregation of MNCS and proliferation of fibroblasts (black arrow) (H & E stain 200× 400×).

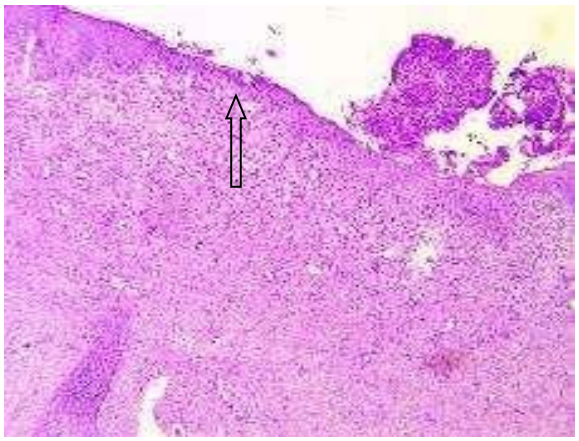

Figure 8: Histopathological section of skin wound from goat in control group at **28** days post wounding incomplete regenerated epithelia with cellular debris (white arrow) (H&E 20×).

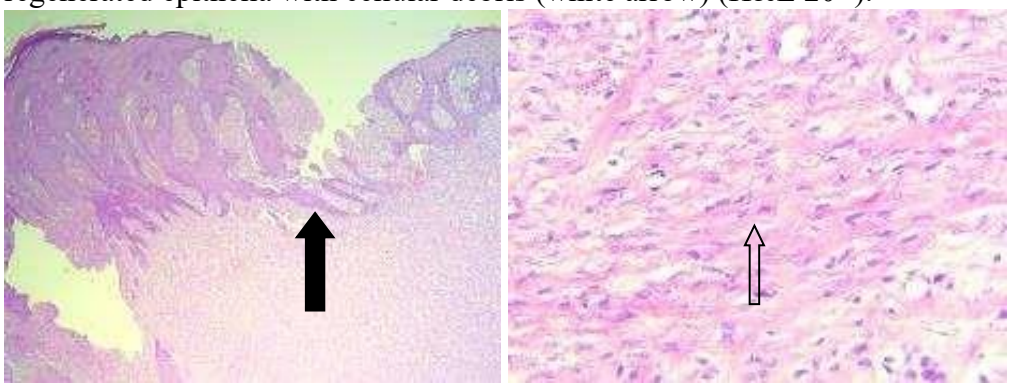

Figure 9: Histopathological section of skin wound from goat in treated group at **28** days post wounding regenerated epithelia of the dermis revealed finger like projection deep in the dermal layer) black arrow), and dense collagen fibers (white arrow) (H&E 20× 40×).

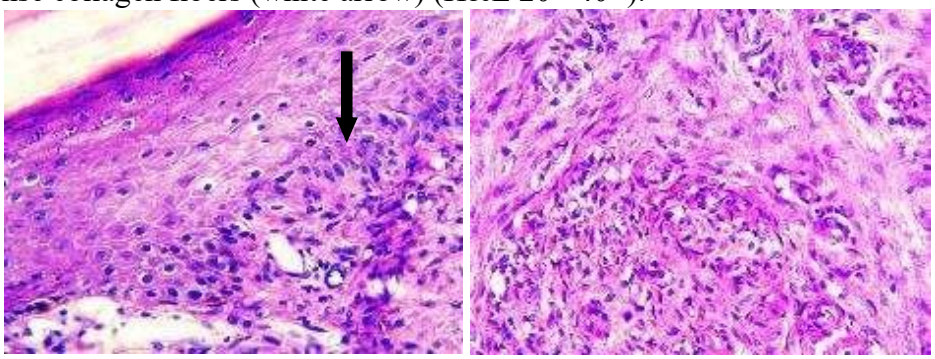

Figure 10: Histopathological section of skin wound from goat in control group at **35** days post wounding, regenerated epithelia with hyperactive basal layer (black arrow) and dense fibrous tissue (H&E 40×).

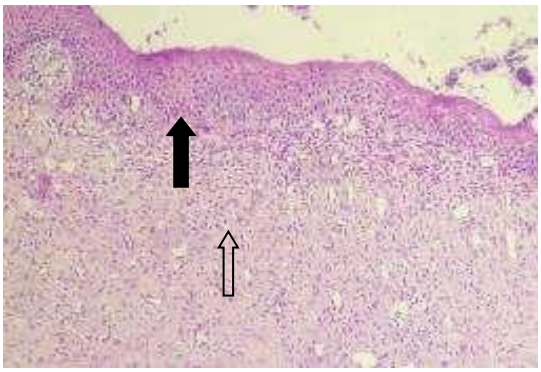

Figure 11: Histopathological section of skin wound from goat in treated group at 35 days post wounding, complete regeneration of the epidermal layer (black arrow) and dense fibrous tissue seen in the dermal layer with MNCS infiltration under the epidermal layer (white arrow) (H&E 20×).

Histopathological results of both groups during the first 7 days of treatment showed hemorrhage, infiltration of inflammatory cell and formation of immature granulation tissue, representing an early reaction to damage in both groups but inflammation was minimum in group B. Same results reported in Al-Falahi (2018) which ADM reveled mild inflammatory reaction and angiogenesis in first 7 days. The early reduction of inflammation in group B might facilitate the progress to the next phase of wound healing (Gangwar *et al*., 2013). However, the treated group had a severe response to wound healing compared to the control group, this agrees with Azari *et al*. (2008) who observed that in goats, the newly formed stroma, or granulation tissue, invades the wound area around four days after trauma. This granular stroma is created by new capillaries, at the same time fibroblasts and macrophages enter the wound area. Fibroblasts create extracellular matrix for cell development, macrophages supply growth factors for fibroplasia and angiogenesis, and blood vessels transport nutrients and oxygen for cell metabolism. Regenerated epithelia, with proliferation of fibrous tissue and fibroblasts with granulation tissues were seen 14 days post wounding in control and treated groups showed that the surrounding wound edges had a gradual regeneration of the epidermis, this in same direction with (AL-Bayati, *et al*., 2013). The underlying granulation tissue increased in mass, the wounds of group B showed partial epithelialization, well-formed collagen, and neovascularization (Lee and McCulloch, 1997).

Regenerated epithelia of the epidermis revealed finger like projection deep in the dermal layer, and dense collagen fibers were seen 28 days post wounding. Cutaneous replacement scaffolds help fibroblast attachment, growing and intrusion which hurry and improve dermal and epidermal restoration. The ECM proteins, involving (collagen, fibrin, fibrinogen, gelatin, elastin, etc., and polysaccharides, especially alginates, hyaluronic acid, cellulose, chitosan, etc.,) this complex mixture have mechanical and biochemical care to adjacent cells and adjust their working in regeneration (ALBayati *et al.,* 2016; Al-Falahi *et al.,* 2018). This result in same direction with (Goodarzi *et al*. 2018), the increasing in thickness of epidermis is directly related to a thickened dermis, and an increase in the constituent elements of the dermis is primary for keratinocyte proliferation. The collagen contained in the dermis can induce cellular regeneration. The sections at 35 days post wounding in control group show's dense fibrous tissue and thick regenerated epithelia with hyperactive basal layer, while treated group were show complete regeneration of the epidermal layer and dense fibrous tissue seen in the dermal layer with MNCs infiltration under the epidermal layer contain many hair follicles, these results may be due to GADPH which consider as autograft substitution which remain contain important factors for healing, that was mentioned by (Keane, *et al*., 2012) which report that ECM hydrogel maintain composition and collagens which is necessary for wound healing proses. GADPH provides optimum condition to strengthen dermal tissue making successful healing outcomes. The matrix of goat skin is processed to keep the three-dimensional structure of the dermal collagen fiber network and to preserve

the biochemistry of the dermis. The collagen constituents of GADPH contain physiological amounts of type I and III collagen found in the extracellular matrix of healing wounds (Gangwar *et al*. 2013). Furthermore, the processing removing cells from the matrix, and fills with growth factors, and glycosaminoglycans, which can be linked with a strong inflammatory profile and quick reabsorption of the hydrogel (Kavros 2012). GADPH outcomes showing farther heling process than control group, due to GADPH provide excellent environment for dermal tissue generation, these results in agreement with (Mohammed & Salih, 2022), who reported that caprine ADM support tissue generation due to its component such as collagens and retain the biochemistry of the dermis.

4. Conclusions

Based on results of our study has shown that the use of GADPH treatment stimulates curing of skin injuries in goats, by an enhancing wound closure in the proliferative phase of the healing. Like many other experiments, this study used a model of acute and uncontaminated wound repair, which does not suggest a different mechanism of impaired healing in chronic and contaminated wounds. GADPH offers the perfect environment to support dermal tissue generation that gives positive healing outcomes with infrequent applications, it had well biocompatibility and could speeding up the wound healing operation.

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Authors' Contribution:

Research is extracted from a doctoral Desertion of the Ph. D for the Student Yasir Salah, University of Baghdad/ Baghdad/ Iraq. Asst. Proof Dr Alawadi (adviser) and Dr. prof. Albayati (secondary adviser) designed all the study experiments. Yasir Salah performed all the experiments, collected all samples of fish and goat skin and experiment animals and conduct the experiment, and wrote the draft of the research under Dr Alawadi and Albayati supervision contributed to check the analyses of the data to the finalize the manuscript for journal submission. All authors approved the final version of the current manuscript for publishing in the respected journal.

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