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FABRICATION OF BIOACTIVE SCAFFOLD FOR WOUND HEALING PROPERTIES FOR THE TREATMENTS OF SKIN LESIONS IN DIABETIC RATS

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ABSTRACT :

Scaffolds, typically made of polymeric biomaterials, provide the structural support for cell attachment and subsequent tissue development. A scaffold is a matrix whose main function is to serve as an anchoring platform for the adhesion of cells and, thus, allow their growth and proliferation, give rigidity to the implanted tissue, and provide an empty volume for vascularization. To find wound healing activities in Scaffold for diabetic Mellitus (DM). In Wistar and Sprague-Dawley (SD) Rats as models of high-fat (HF) diet-induced obesity have not yet been adequately evaluated for 17 weeks, to check the anti-diabetic activity for diabetic Mellitus patients and treatment for wounds and scars of diabetic Mellitus.

1. INTRODUCTION :

Nearly 20% of people with diabetes mellitus (DM), a chronic metabolic disorder that affects more than 340 million people worldwide, acquire diabetic wounds [1]. Non-healing and poorly healing wounds pose a significant threat to world health due to the increased prevalence of DM and the lack of preventative measures [2]. Acute wounds heal in phases, and within four weeks, clear indications of healing appear. Healing does not become evident in chronic wounds within four weeks and chronic wounds do not progress through the stages of healing normally. One could assert that the wound healing process is influenced by elements at the wound site, systemic mediators, the nature of the damage, or any underlying diseases [3]. Only 1% of the roughly 500,000 plant species in the world have had their phytochemical makeup examined, indicating a significant opportunity for the finding of novel bioactive compounds [4]. Nearly 80% of the populace of developing nations, as well as economic powerhouses like China and India, uses traditional medicine to address a variety of diseases [5, 6]. In chronic wound therapy, such as for diabetic chronic ulcers, where there are few effective treatment options and poor healing results, wound dressings are gaining significant, widespread focus. Therefore, it would be very advantageous to create a successful wound dressing

treatment [7, 8]. In order to encourage cell proliferation and adhesion, protect the wound from harmful environments, and prevent secondary wound damage, the ideal wound dressing scaffold should function similarly to skin tissue and resemble it structurally. This will give the dressing mechanical strength and speed up wound healing [8].

Wound dressings with a bi-layered model have been developed in an effort to mimic the construction and operation of skin. Examples include OASIS (porcine a cellular lyophilized small intestine tissue) and BiobraneTM (silicone, nylon mesh, and collagen) [9]. The scaffolds have a tridimensional structure with connected pores in wounds and serve as a barrier against bacterial infection and water loss. However, there are still some drawbacks to these scaffolds, including cost-related issues, infection risk factors, and impermanent coverage [10, 11]. On the other hand, the process of healing a wound entails a combination of activities involving the interaction of proteins, growth factors, and extracellular matrix processes as well as blood cells (platelets, fibroblasts, erythrocytes, macrophages, leukocytes, etc.)[12, 13].The need for better materials to accomplish hemostasis is therefore extremely high. By quickly attaining hemostasis through timely application of a potent hemostatic material, blood loss may be effectively controlled, hastening the healing of wounds and lowering mortality. Large protein collagen is broken down into smaller, functional peptides by enzymes, which have been shown to have wound-healing properties[14, 15].Collagen peptides have a high bioavailability, which enables the gastric enzymes to digest, ingest, and transport them. Collagen peptides' chemotactic characteristics encourage cell migration and proliferation, which is crucial for wound healing [16, 17].

According to their source, the biomaterials presently used as matrices for wound repair can be divided into natural and synthetic kinds [18].Natural biomaterial has been used extensively due to its attributes of simple degradation, remarkable biocompatibility, decreased inflammatory responses, and non-immunogenic nature. Suggested are collagen (COL) [19].One of the primary extracellular matrix (ECM) protein components, COL, may control the phenotype of cells and alter the physicochemical properties of the scaffold [20].Additionally, COL is said to be the vehicle for numerous cross-links thanks to the chemical groups that are abundant in the branches of the molecular structure drugs [21].Unfortunately, the use of pure COL scaffolds is constrained by their poor mechanical properties, unstable porous structure under wet circumstances, and absence of biological activity [22].Furthermore, adding Graphene oxide (GO) nanosheets can significantly increase the mechanical resilience of GO-related polymeric scaffolds [23].After being inserted into the body, scaffolds containing GO are broken down by immune cells' peroxidase, which leads to their ultimate excretion in the form of faeces and urine [24].The remarkable biocompatibility, biodegradability, and mechanical properties of GO make polymeric scaffolds incorporating it ideal for tissue engineering uses [25].Polymer-based wound dressing materials have received a lot of focus in the treatment of chronic wounds, particularly diabetic wounds. These bandages exhibit a number of intriguing qualities that are useful for treating chronic wounds. High porosity and swelling capacity, adequate water vapour transmission rate (WVTR), the ability to provide moisture and a warm environment to hasten the healing process, gaseous permeation, excellent antimicrobial properties, excellent mechanical performance, and the capacity to deliver bioactive agents are all characteristics of ideal polymeric dressings [26, 27,28].

Numerous man-made and natural polymers have been investigated for use in creating these frameworks. A critical stage in ensuring the success of the scaffold is the appropriate selection of the material used to create it. Due to their interactions with different cells and lack of an immune response, natural polymers were among the first scaffold materials used in

clinical practice; however, synthetic polymers were subsequently used because of their lower cost and superior functionality. (regardless of the possible immune response to them or toxicity)[29].Collagen, chitosan, fibrin, gelatin, hyaluronic acid (HA), and silk are some examples of naturally occurring polymers that have been utilized as tissue supports. Biomaterials, both manufactured and natural, were used to create scaffolds, including nanofibers, hydrogels, mats, 3D structures, sponges, foams, membranes, and nanogels.[29] Wound healing structures have been created using a variety of techniques up to this point, including extrusion, molding, freeze-drying, rapid prototyping, and electro spinning. Due to its ease of use, low cost, flexibility, and scalability, the electro spinning method is still among the most popular of these [30].The following characteristics are essential for a perfect scaffold for wound healing applications: superb physiological support for cell adhesion, proliferation, and/or differentiation. Additionally, a scaffold must have a high porosity, a high surface area to volume ratio, an interconnected geometry, and the flexibility to conform to the contour of the wound. The scaffold should ideally have a degradation rate that corresponds to the time needed for wound repair and be both biocompatible and biodegradable. The scaffold should also keep a moist environment because this is necessary for providing signals that promote cellular adhesion, growth, and migration as well as angiogenesis, granulation tissue formation, and re-epithelialization [31].

The immune system changing, a persistent inflammatory process being present, tissue hypoxia, epidermal cell dysfunction, and an impairment of the angiogenesis process are just a few of the factors that can impede recovery in diabetes. Increased reactive oxygen species (ROS) production causes oxidative stress, which prolongs the inflammation phase and, as a result, modifies the proliferative phase. ROS also cause the production of cytokines and matrix metalloproteases (MMPs), which are other by products of oxidative stress [32].On the other hand, several studies looking for novel therapeutic suggestions for the treatment of such wounds have focused on the incorporation of bioactive agents with healing, anti-inflammatory, and/or antioxidant potential in various types of formulations [33].Damage to tissues, oxidative stress, and delayed wound healing have all been linked to imbalances in the production of free radicals and antioxidants. Thus, a major method for treating chronic wounds could be the elimination of Reactive oxygen species (ROS) [34].

Homeostasis, inflammation, proliferation, and remodelling are the four overlapping stages of the healing process, and a variety of growth factors, enzymes, and cytokines are also involved. These factors have a significant impact on the synergistic modulation of relevant cell activities[35, 36,37,38].Platelets are then activated when they come into contact with the vascular sub-endothelial matrix, homeostasis, and coagulation phases after damaged blood vessels rapidly contract after injury and a blood clot forms to halt exsanguinations brought on by vascular damage [39, 40].Growth factors and cytokines, such as (i) insulin-like, (ii) platelet-derived, (iii), transforming, and (iv) epidermal growth factors, are abundant in platelets. These substances stimulate and draw neutrophils, which then draw macrophages, endothelial cells, and fibroblasts, serving as repair agents in the wound healing cascade [41].The next phase is the inflammatory one, which begins with

the early inflammatory reaction that starts during the late coagulation phase and ends soon after. In the vicinity of the lesion, where they serve primarily as wound cleaners, this phase causes neutrophil aggregation. Within 24 to 36 hours following injury, bacteria and platelet products release a variety of chemo-attractive chemicals that entice neutrophils to the wound site where they can phagocytose the bacteria and avoid infection. Neutrophils are eliminated by apoptosis once all contaminating microorganisms have been eliminated, and wound macrophages resume the phagocytosis process 48–72 hours later. Compared to neutrophils, macrophages live longer and serve as crucial regulatory cells, providing a plentiful supply of powerful tissue growth hormones, fibroblasts, and other cells [42].Scientists have been experimenting with different combinations of the tissue engineering triad-growth factors, stem cells, and scaffolds—to create better and more affordable methods for wound healing and repair [43].

Acute wounds can also be brought on by a variety of environmental factors, such as exposure to radiation, chemicals, or severe temperature changes, which can result in an organism entering the body and infection. Depending on their height, depth, and location, they can be categorized [44].The hemostatic function of creating blood clots inside the cut area. It only requires a brief time, from a few minutes to a few hours after the injury. After a few hours, the second phase, which is in charge of the defensive function, begins and lasts for about one week. Leukocytes (neutrophils) and monocytes must migrate from blood vessels into the injured tissues in order to differentiate into macrophages. Additionally, it is at this time that lymphianogenesis and angiogenesis begin. The third period begins around five days after the injury and lasts for the next 10 to 14 days. As fibroblasts, myofibroblasts, and keratinocytes continue to proliferate to create the dermal and epidermal layers, respectively, lymphianogenesis and angiogenesis continue to assist this process. The concluding (fourth) phase is now complete [45, 46].

1.1 High Fat Diet:

Obesity and metabolic complications have become epidemics, with animal models like Wistar and SD Rats being used to study these pathologies. A 17-week trial involving standard and high-fat diets showed that HF diets accelerated weight gain and body composition, highlighting the need for further researc

Fig1: High Fat Diet Feed Animal

1.2 Clinical Strategies For Diabetes Wound Treatment :

Diabetes-related wound treatment involves a multidisciplinary approach including glucose control, wound care, infection management, and offloading. Tight glycemic control is crucial to prevent wound healing and infection. Effective wound care involves cleaning, debridement, and dressing to maintain a moist environment. Prompt identification and management of infection is essential, potentially involving antibiotics or antimicrobial therapies. Offloading, using devices to relieve pressure, promotes healing. Advanced therapies like growth factors, stem cells, and hyperbaric oxygen therapy may also be used.

1.3 Role Of Biomaterials In Diabetes Wound Treatment And Tissue Regeneration :

Biomaterials are crucial in diabetic wound treatment and tissue regeneration. They provide a scaffold for cell growth and promote cell growth through materials like collagen, hyaluronic acid, and alginate. They also control drug delivery by releasing drugs over time, providing sustained therapeutic effects. Biomaterials can also reduce inflammation at the wound site with anti-inflammatory properties like curcumin and silver nanoparticles. Additionally, they improve wound closure and reduce scarring with silicone sheets and hydrocolloid dressings. However, further research is needed to develop and optimize biomaterials for these applications.

1.4 Development And Changes Of Degradable Biomaterials :

Biodegradable biomaterials have evolved from initial coverings to cell culture matrix with bionic function, serving as scaffolds for wound healing and promoting cell growth. The first generation was based on natural materials, but later synthetic biomaterials were developed. Advances in 3D printing and manufacturing technologies have enabled the production of complex structures and scaffolds with precise geometry and mechanical properties. The use of degradable biomaterials has expanded beyond tissue engineering to include drug delivery, wound healing, and medical devices.

1.5 Regenerated Scaffold Or Environment :

Regenerated scaffolds or environments are biomaterials used to support tissue regeneration, mimicking the structure and function of the regenerated tissue. They are used in tissue engineering, wound healing, and drug delivery. Advances in biomaterials have enabled more complex and precise scaffolds, improving tissue regeneration success rates. Challenges include balancing mechanical strength, biocompatibility, and degradation rate, and understanding the interactions between biomaterials and host tissues. Future research aims to develop more advanced scaffolds with enhanced functional properties.

1.6 Restore The Natural Structure Of ECM :

The extracellular matrix (ECM) is crucial for tissue structure and function in biological systems. Scaffold-based approaches use biomaterials to restore the ECM's natural structure, while combination approaches use multiple strategies. Understanding the native ECM structure of the regenerated tissue, including its composition and mechanical properties, is essential. Preclinical and clinical studies are necessary to evaluate the safety and efficacy of these restoration approaches.

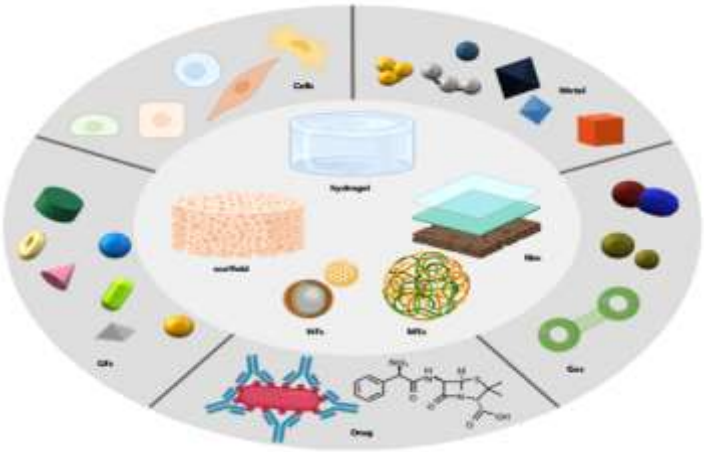


Fig.2 The combination of composite biodegradable biomaterials.

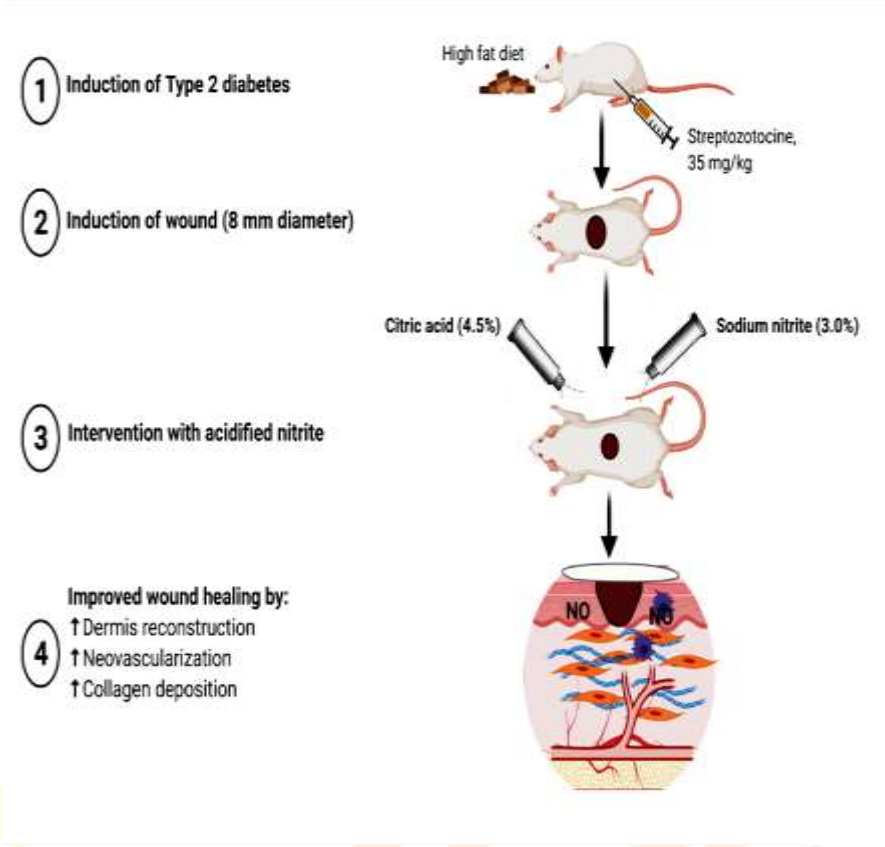


Fig. 3 Graphical abstract of Experimental design.

1.7 Biomaterial And NF-B Signalling Pathway In Wound Healing :

NF-κB is a transcription factor that is crucial in wound healing, activating genes that promote inflammation, angiogenesis, and tissue remodeling. However, prolonged activation can lead to chronic inflammation and impaired healing. Biomaterials can modulate the NF-κB signaling pathway, controlling the release of anti-inflammatory agents and delivering growth factors and cytokines. They can also mimic the extracellular matrix structure, providing a scaffold for cell attachment, proliferation, and differentiation, promoting tissue regeneration and remodeling, ultimately restoring tissue function.

2. MATERIAL AND METHODS:

The study involved pathogen-free adult male Wistar strain albino rats, acclimatized to standard laboratory conditions, and randomized into four experimental groups. The rats were fed a high-fat diet for 12 weeks, with the control group being a normal standard diet, the diabetic group being a high-fat diet, and the diabetic group being a high-fat diet.

3. Materials And Reagents :

The TRIzol reagent, sodium acetate, chloroform, ethanol, Lowry's reagent, Takara PCR kit for RT PCR, sodium hydroxide, disodium carbonate, copper sulphate, sodium potassium tartarate, Folins reagent, bovine serum albumin, forward and reverse primers were used in a mixture of phosphate buffered saline, pH 8.0, and TBST.

In Vitro Cell Biocompatibility Of The GO-COL And GO-COL-NAC Scaffolds:

The study used ATCC HaCaT cells from the USA for cell proliferation and NIH 3T3 fibroblasts for cell migration. The cells were maintained in a growth medium enriched with DMEM, high glucose, and penicillin/streptomycin. After 24 hours, the media was collected, centrifuged, and filtered.

In Vivo Wound Healing Studies :

A study involving 54 adult Wistar rats was conducted to assess their biological activity in vivo. The rats were diabetic and given alloxan intraperitoneally. After 60 days of diabetes, the skin was marked off with a metal punch. Treatment with HC and HCF accelerated wound closure, with HCF allowing faster healing. HCF also produced more fibroblasts and collagen, demonstrating the re-epithelialization of wounds.

Wound Creation :

Pentobarbitone sodium was injected into rats to anesthetize them, and they were then shaved, washed, and incision made. After recovery, they were divided into three groups and treated with different formulations and solutions for 14 days.

Fig 5: Xylazine injection and ketamine injection

1.7 Photographs Of Wounds And Calculations Of The Percentage Of Wound Contraction :

Wounds were photographed on various days and measured at specified intervals. The wound area was calculated using planimetric calculations. Percent wound contraction was used to express findings, and Wilson's algorithm was used to determine values. The wound area was represented as percentages of the 0 day readings.

$$\% \text{ Wound Concentration} = \frac{\text{Day wound area – unhealed wound}}{\text{0 day wound area}} \times 100$$

1.8 Collection Of Tissue :

The animals were killed on days 3, 7, 11, and 14 with diethyl ether, and the healing tissue was removed. Three sections were cut, one stored in RNA for real-time PCR, and the other frozen. Tissue homogenate was made using a motor homogenizer and incubated for four hours at 4°C.

1.9 mRNA Expression Studies :

Total RNA was extracted using Ribozol TM RNA extraction reagents. CDNA was created using a cDNA synthesis kit, standardized for each gene using glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Real Time PCR was performed on target and reference genes, and a dissociation curve was created to evaluate amplified product specificity.

1.10 Staining With Haematoxylin And Eosin :

The study involved preserving granulation tissue and healing in formalin, embedding it in paraffin, and cutting it into 5mm thick slices. The sections were stained with H&E and rated by an expert pathologist blindly. The scoring process was based on the presence of cell accumulation or immature tissue.

1.11 Antioxidant Activity :

The study found that animals treated with both formulations for 14 days showed higher MDA concentrations in lesions, indicating an increase in oxygen reactive oxygen species within 14 days of lesion induction. The fraction richer in flavonoids may have potentiated this effect.

1.12 Macroscopic Evaluation :

Following the surgical operation, the lesions were photographed on Day 0, Day 2, Day 6, and Day 14. The area of the wound and its contraction were then calculated from the photos using Image software.

1.13 Microscopic Evaluation :

Following euthanasia, wound tissues were collected, stored in formalin, and paraffin-stained using haematoxylin and eosin to examine wound healing.

1.14 Evaluation Of Antioxidant Action :

Malonic dialdehyde (MDA) was quantified using fluorimetry to measure lipid peroxidation. Tetramethoxypropane was used for calibration. SOD activity was measured using Oyanagui's technique, and GPx activity was measured using Sinet's method.

1.15 Analysis Of Collagen Using Real-Time Polymerase Chain Reaction :

The mRNA expression levels of COL Type I and COL Type III of NIH-3T3 fibroblasts were assessed using real-time RT-PCR. Trizol reagent was used for extraction, and Revert Aid First Strand cDNA Synthesis Kit was used for reverse transcription. The PCR product was quantified using real-time PCR equipment.

1.16 Histological Analysis :

After surgery, rats were slaughtered and tissues were collected. Hematoxylin-Eosin and Masson trichrome staining were used to assess epidermis, collagen, and new tissue development, while immunohistochemistry was used to examine vascular endothelial cells.

1.17 Detection Of Antioxidant Stress Level :

RT-PCR analysis assessed antioxidant kinase expression in skin wound samples using 95°C for 5 minutes, 10 seconds, and 60°C for 30 seconds (40 cycles)

1.18 Isolation of cDNA from RNA via Conventional PCR :

1. Prepare the following mixture in a microtube

Reagent	Volume
Oligo dT Primer (50 µM)	1µl
Or Random 6 mers (50 µM)	Or 1 µl (0.4-2 µl)*
dNTP Mixture (10 mM each)	1µl
Template RNA	total RNA: <5 g*2polyA+ RNA: <1 µg
RNase Free dH O	1µL
Total	**10µl**

2. Incubate for 5 min at 65°C, then cool immediately on ice.

3. Prepare the reaction mixture in a total volume of 20µl

Reagent	Volume
Template RNA Primer Mixture (from step 2)	10 µl
5X PrimeScript Buffer	4 µl
RNase Inhibitor (40 U/µ)	0.5 µl(20U)
PrimeScript RTase (200 U/µl)	1.0 µl (200 U)
RNase Free dH_2O	1µl
Total	**20 µl**

4. Mix gently

5. Incubate the reaction mixture using the following conditions.

30°C10 min (required when using Random 6mers) 42°C (50°C) *4 30-60 min

6. Inactivate the enzyme by incubating at 95°C for 5 min, 5 then cool on ice.

For cDNA synthesis, use 0.4 µl for random 6 mers and 2 µl for reverse transcription. Use less than 1 µg of total RNA for 1st strand cDNA templates. React at 42°C, 50°C for reverse transcription reactions, and 70°C for 15 min for longer cDNAs to minimize cDNA damage.

Storage and Stability :

Guaranteed for 12 months in a constant temperature freezer at -20°C protected from light. For convenience, this supermix can be set4°C short-term or refrozen up to ten times.

1.19 Kit Contents :

1. Taq Universal SYBR Green supermix is a 2x concentrated, ready-to-use reaction master mix optimized for dye-based quantitative (qPCR) on any real-time PCR instrument (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start iTaq DNA.

2. Polymerase, dNTPs, MgCL, SYBR Green dye, enhancers, stabilizers, and a blend of passive reference dyes (including ROX and fluorescein).

1.20 Instrument Compatibility :

1. The supermix is compatible with all Bio-Rad and ROX-dependent Applied Biosystems real-time PCR instruments and with the Roch.

2. LightCycler LC480, QIAGEN Rotor-Gene Q. Eppendorf Mastercycler EP realplex, and Stratagem Mx real-time PCR systems.

1.21 Reaction Mix Preparation and Thermal Cycling Protocol :

1. Thaw iTaqM Universal SYBR Green super mix and other frozen reaction components to room temperature. briefly to collect solutions at the bottom of tubes, and then store on ice protected from light.

2.Prepare (on ice or at room temperature) enough assay master mix for all reactions by adding all required components except the template according to the following recommendation.

Table 3. Reaction setup

Component	**Volumeper20µlReaction**	**Volumeper10µlReaction**	**Final Concentration**
Taq™ Universal SYBR Greensupermix (2x)	10 µl	5µl	1x
Forward and reverse primers	Variable	Variable	300-500 nM each
DNA template	Variable	Variable	cDNA: 100 ng-100 fg Genomic DNA: 50 ng-5 pg
H_2O	Variable	Variable	____
Total reaction mix volume	20 µl	10 µl	____

Scale all components proportionally according to sample number and reaction.

Real-Time PCR System	Setting/ Mode	Polymerase Activation& DNA Denaturation at 95 °C	Denaturation at 95°C	Annealing Extension + Plate Read at 60°C	Cycles	Melt-Curve Analysis
Bio-Rad CFX96,CFX384" CFX96 Touch", CFX384 Touch CFX Connect systems	SYBR®only	20-30 sec for CDNA	2-5 sec	15-30 sec		
Bio-Rad iQ 5, MiniOpticon, Chromo4,Myio	Standard	Or	10-15 sec	15-30 sec		
ABI 7500, StepOne, StepOnePlus, 7900ht,and ViA7	Fast Standard	2-5 min for gDNA	1-3 sec 15 sec	20-30 sec 60 sec	35-40	65°C-95°C-0.5°C increment 2-5 sec/ste (or use instrument default setting
ABI 7300 and 7000	Standard		15 sec	60 sec		
Roche Light Cycler 480	Fast Standard		2-5 sec 15 sec	15-30 sec 60 sec		
QIAGEN Rotor-Gene and Stratagene Mx series	Fast		2-5 sec	15-30 sec		

Table 4. Volume

Mix the assay master mix thoroughly, dispense equal aliquots into tubes or plates, and vortex for 30 seconds. Add DNA samples and DNase-free HO, spin, and collect the reaction mixture. Program thermal cycling protocol on a real-time PCR instrument, load tubes or plates, start the run, and perform data analysis according to instrument-specific instructions.

Recommendations for Primer Design:

The iTaq™ Universal SYBR Green supermix and qPCR cycling protocols are optimized for assays with 60°C primers, designed using Primer 3 or Pr Express software. For optimal efficiency, target amplicons 70-150 bp, with longer annealing extension times for cons.

Quality control:

Taq™ Universal SYBR Green supermix demonstrates >90% PCR efficiency and linear resolution over seven Stringent specifications are maintained to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

Table ; 5 QUANTIFICATION OF cDNA:

Sample Reader	Well Id	Location	260	280	260/280	ng/µL
1	SPL1	B2	0.081	0.039	2.092	65.109
1	SPL2	B3	0.078	0.037	2.086	62.362
1	SPL3	C2	0.341	0.196	1.738	272.819
1	SPL4	C3	0.686	0.357	1.92	548.888
1	SPL5	D1	0.017	0.003	4.841	13.278
1	SPL6	D2	0.017	0.003	6.34	13.459

1.22 In Vivo Rat Wound Healing Model And Histopathological Evaluation :

The study investigated the effects of a 50% ethanol extract of Bacopa monniera (BME) on wound healing in rat wound models. The extract was administered orally for 10 days or longer, and its effects on wound breaking strength, wound breaking strength, and skin histology were examined[68].

1.23 Experimental Design :

The study aimed to establish an experimental model for STZ-induced diabetic rats, dividing them into two groups: Control and diabetic rats after 20 weeks.

1.24 Rat Body Weight Was Examined Throughout The Experiment (N=6) :

This study uses age-related variations in rats' body weights to summarize postnatal development phases. It highlights the impact of animal age and body weight on gene expression, metabolic parameters, and medication metabolism. Proper reporting of animals' age, body weight, and developmental stage is crucial for accurate animal testing and understanding human biology[69].

Establishing the experimental model ;

Fig 6: Normal Diet fed rats (N=6) Group 1

Fig 7: High fat diet fed rats (N= 6) Group 2

Five-week-old male Wister albino rats weighing 180−220 g divided in to two groups respectively, Group 1 – Control, Group 2 – High Fat diet (HFD)

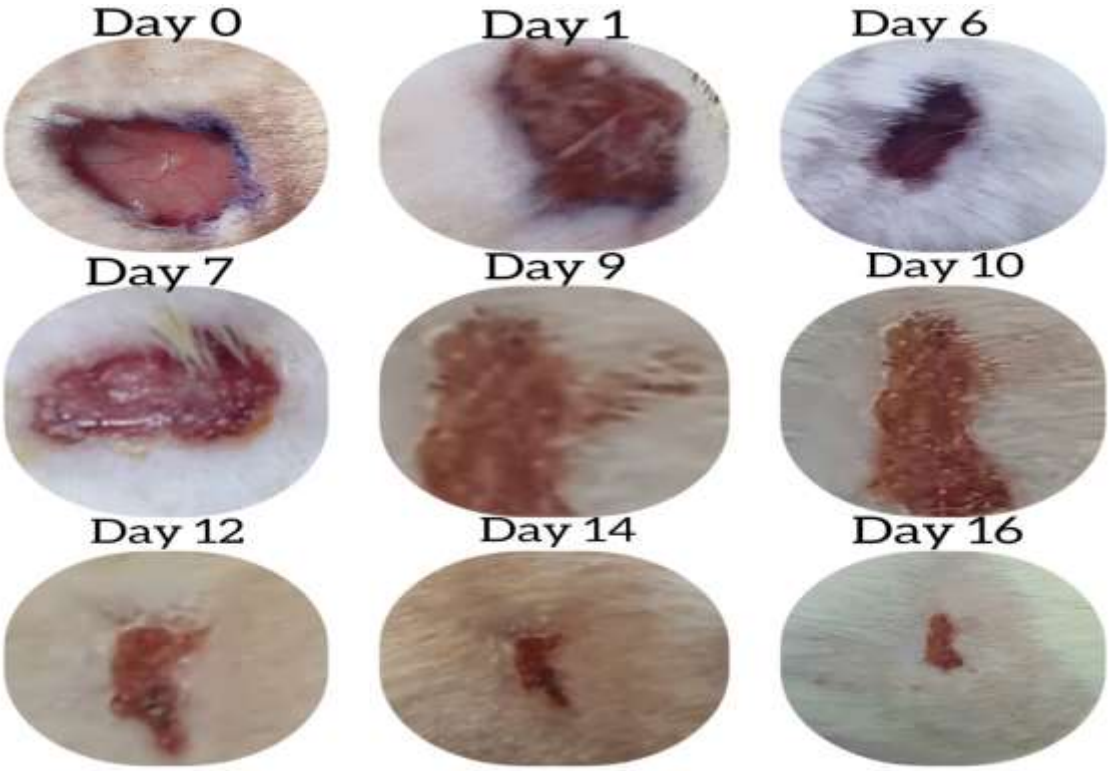
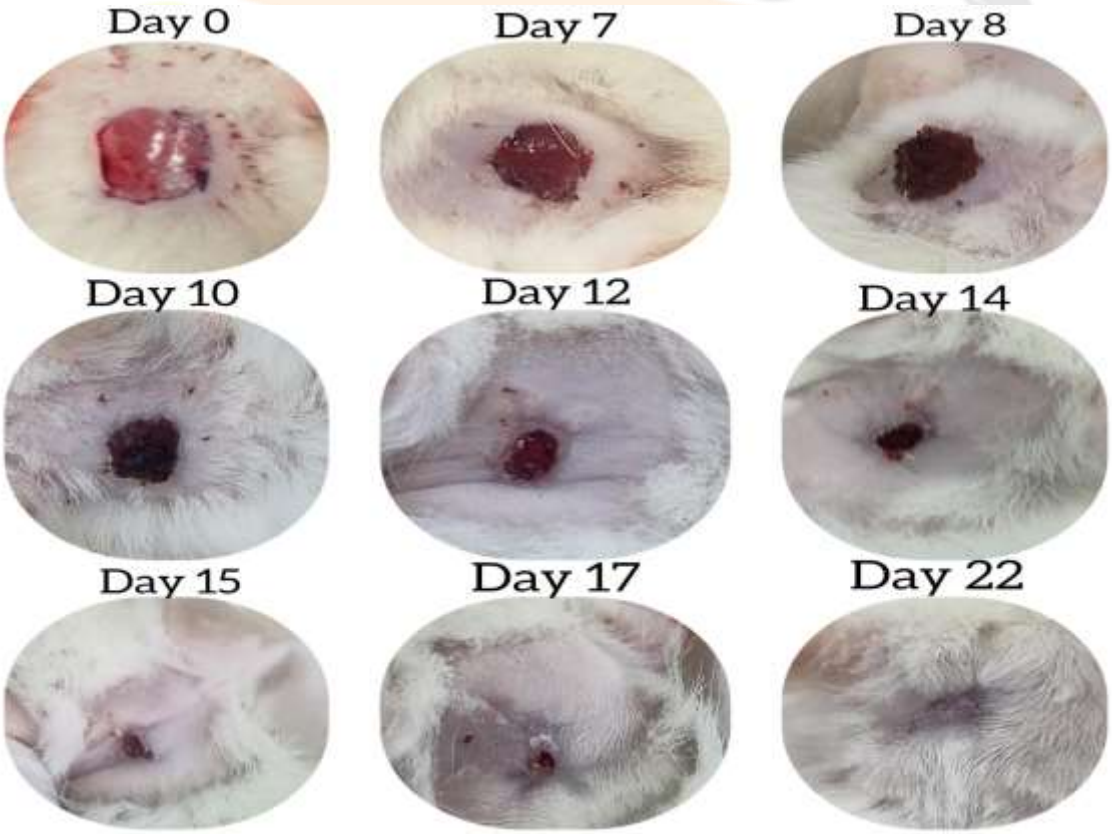


Fig: 8 Photography of wound creation and closure



Treated: **Fig: 9 Diabetic Wound Created Rat**

4.27 Evaluate The Major Signalling Pathways Involved In Wound Healing Mechanism By qPCR Analysis :

Wound healing mechanisms involve various signaling pathways, including growth factors, cytokines, and other molecules. qPCR analysis is a valuable tool for quantitatively analyzing gene expression and signaling pathways involved in wound healing. Major signaling pathways include TGF-β, VEGF, MAPK, and ECM pathways. TGF-β stimulates the production of extracellular matrix proteins, promoting fibroblast differentiation and migration. VEGF stimulates blood vessel formation during wound healing. MAPK regulates cellular proliferation and migration. ECM proteins, such as collagen, elastin, and fibrin, provide structural support to the skin. qPCR analysis helps researchers identify key regulators and develop new therapeutic strategies.

1.28 Relative mRNA expression levels of nuclear factor kappa-B (NF-κB), GLUT4, interleukin-1β (IL-1β) and COLA1 in heat tissue samples assessed by qRT-PCR. Data are mean ± SEM (n 6). *p < 0.05 Control Vs DN group :

The study analyzed the relative mRNA expression levels of NF-κB, GLUT4, IL-1β, and COLA1 in heat tissue samples using qRT-PCR. The data showed differences between the Control and DN groups, with a p-value of less than 0.05. GLUT4 is a glucose transporter gene that may be altered in diabetic and insulin-resistant conditions. NF-κB, a transcription factor, regulates inflammation and immune response. IL-1β, a cytokine, is linked to inflammatory conditions like rheumatoid arthritis and inflammatory bowel disease. COLA1, a collagen type, may be altered in connective tissue disorders. The study suggests potential changes in the extracellular matrix architecture in heat tissue samples.

1.29 Excision Wound Model :

Rats were anesthetized with ketamine hydrochloride and xylazine, and ketoprofen was given before surgery. Skin was sterilized, and a lesion was created in the posterior dorsal region. Biomaterials were applied, and animals were housed individually in disinfected cages to prevent infection

4.30 Determination of Wound Retraction Percentage :

Daily wound closure analysis was conducted in each treatment period group, with wound area measured using Adobe Photoshop C5-version 5. Wound retraction area was calculated using a formula, expressed as mean ± standard deviation.

4.31 Liver histological analysis :

Zhang et al. (2004) used Tellyesniczky's solution to fix liver tissues from rats, which were then washed, dehydrated, and embedded in paraffin wax. Skin samples were fixed with alcohol, formalin, and acetic acid, and analyzed for blood vessels and collagen fiber deposition. Ten photomicrographs were analyzed under 40x magnification.

4.32 RNA Extraction and Reverse Transcription Quantitative PCR (RT-qPCR) :

The study involved collecting and freezing lesion samples from animals, macerating them, extracting RNA, and synthesising cDNA. The RNA samples were treated with DiNase I to prevent contamination. Quantitative PCR was performed to evaluate gene expression in Rattus norvegicus. Cts values were determined using the SYBR Green kit. The mRNA levels were normalized by GAPDH and expressed relative to the Cts mean of each group. Relative gene expression profiles were calculated using the ΔΔCT value.

Table 6: Expression of gene:

IL-6	5′- CCG GAG AGG AGA CTT CAC -3′
	5′- TCC ACG ATT TCC CAG AGA -3
TNF-α	5′- TCA GTT CCA TGG CCC AGA C -3′
	5′- GTT GTC TTT GAG ATC CAT GCC ATT -3
NF-κB	5′- GTT GTC TTT GAG ATC CAT GCC ATT -3
	5′- AGC CCC TAA TAC ACG CCT CT -3
IL1-β	5′-TTGAAGTTGACGGACCCCAA-3′,
	5′-TGCTGCTGCGAGATTTGAAG-3′
GAPDH	5′- CAT CCG TAA AGA CCT CTA TGC CAA C-3′
	5′- ATG GAG CCA CCG ATC CAC A -3′

4.33 Statistical Analysis :

Statistical analysis was conducted using GraphPad Prism 5.01 software, using Kruskal-Wallis, Dunn, and ANOVA for nonparametric and parametric data, with p-values < 0.05 considered statistically significant.

5. RESULT :

Wound healing in rats involves a series of events including inflammation, proliferation, and remodeling to restore tissue integrity and function. The rate of wound closure, restoration of tissue integrity, reduction of inflammation, prevention of infections, quality of scar tissue, and improvement in quality of life are all key outcomes. The type and size of the wound, age, and health of the rat also influence the healing process. In cases where wound healing is delayed or unsuccessful, the rat may experience persistent inflammation, wound dehiscence, increased pain, greater infection risk, and reduced quality of life. Further interventions may be necessary to promote successful wound healing.

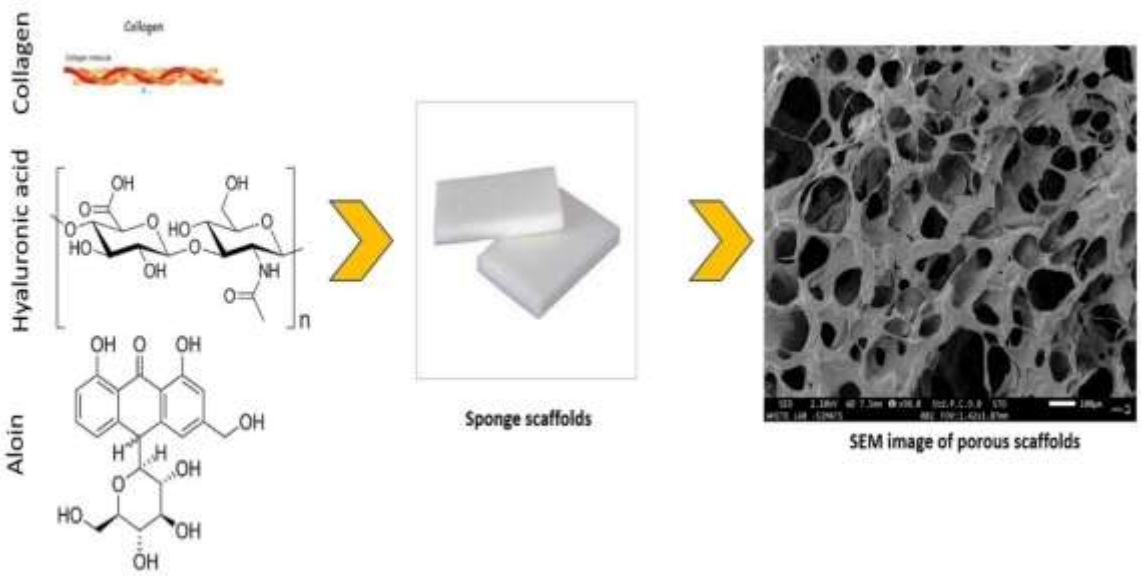


Fig. 10 Preparation and characterization of Bio-Based Natural scaffold sponge Materials

To establish the experimental model for STZ induced diabetic rat models.

Five-week-old male Wister albino rats weighing 220−240 g divided in to two groups respectively,

Group 1 – Control

Group 2 – STZ induced Diabetic Rat

Group 2 – STZ Diabetic rats with Biomaterial

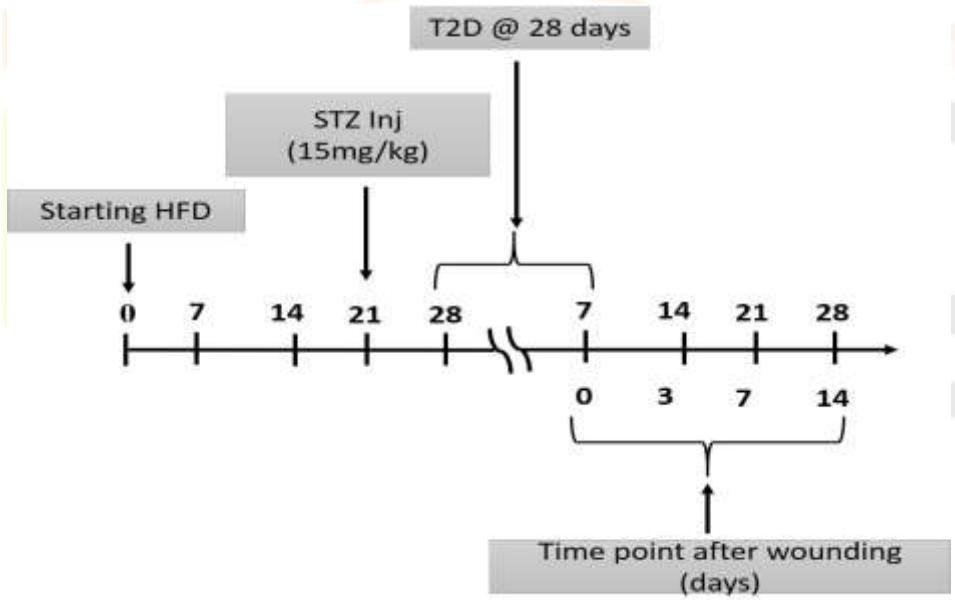


Fig .11

Time point after wounding days

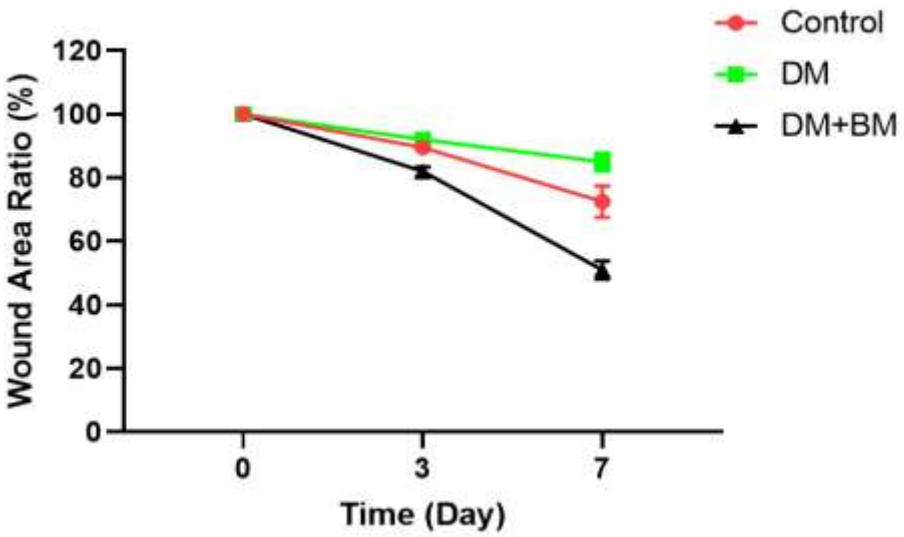
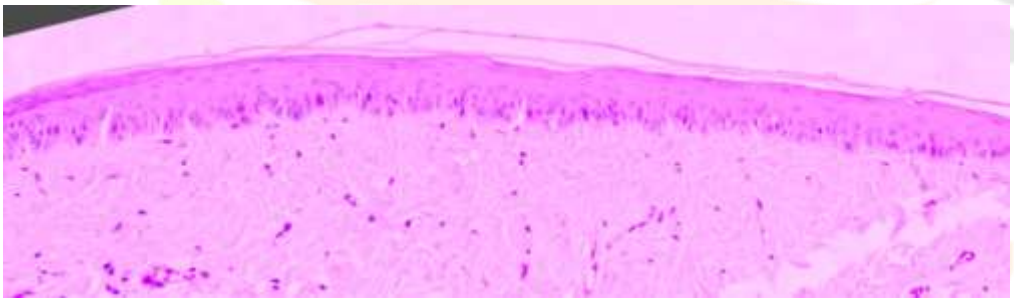


Fig.12 Wound Area Ratio

The wound area ratio of three groups, P <0.05, (one-way ANOVA followed by Bonferroni's multiple comparison test).

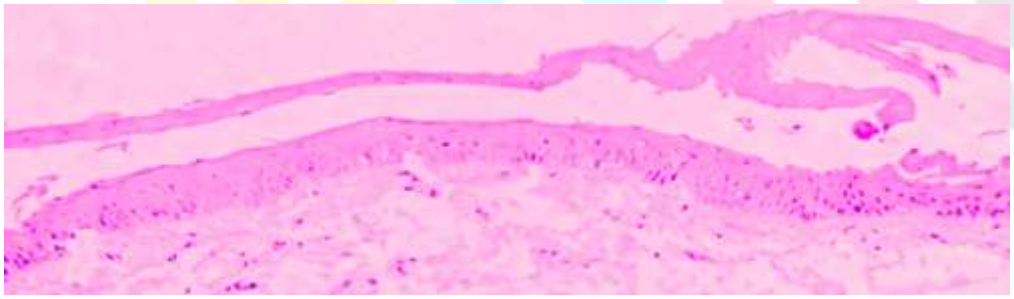
Control

0 W



0 W

Healing



7 W

Defect

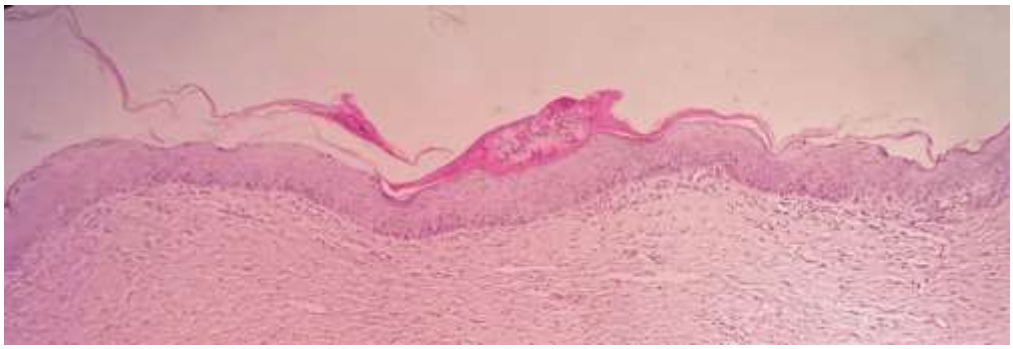


Fig.13 Histopathology Result

Histopathological evaluation of wound constriction observation from the control and diabetic rat model using H&E staining

Histopathological evidence of the skin closer to the control and diabetic rat after the wound was created (7 weeks). Microscopic view of rat skin stained with hematoxylin and eosin. (**a**) control rat and (**b**) wound model created in diabetic rats. (**c**) Wound model with biomaterial scaffold patched diabetic rat. (scale bar: 50 μm).

Evaluate the major signaling pathways involved in wound healing mechanism by qPCR analysis

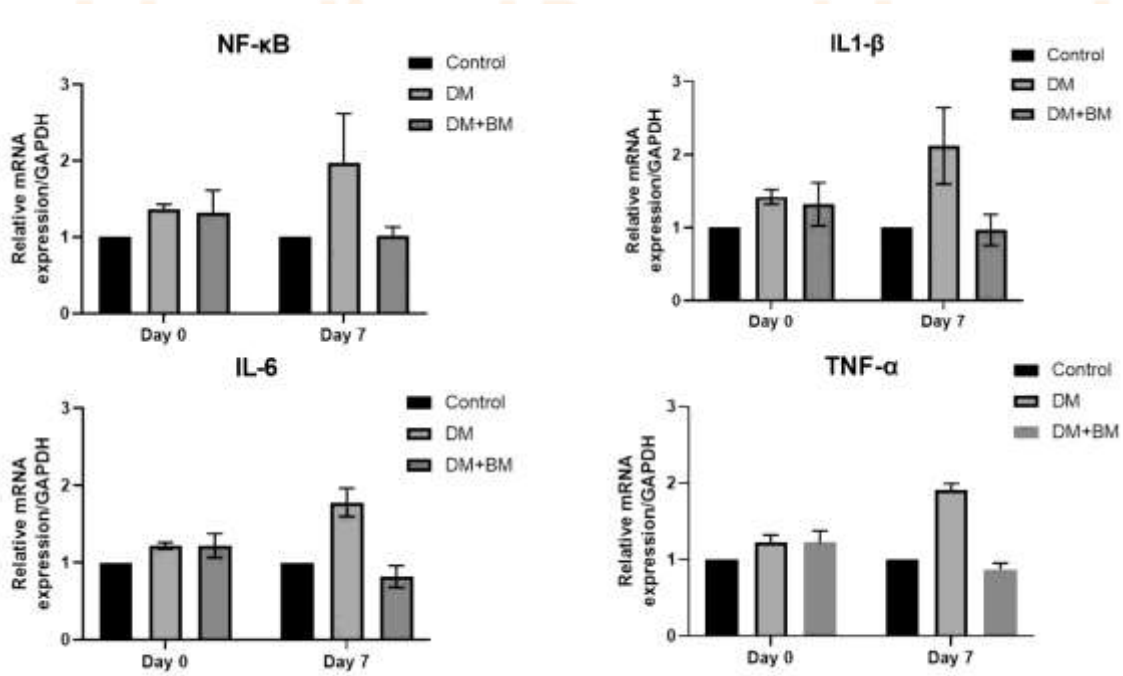


Fig.14 mRNA Expession

Relative mRNA expression levels of nuclear factor kappa-B (NF-κB),interleukin-1β (IL-1β) and IL-6 and TNF-a in Skin tissue with control and experimental groups assessed by qRT-PCR respectively, Data are mean ± SEM (n= 6). *p < 0.05 Control Vs experimental group.

2.DISCUSSION:

Wound healing in rats is a growing research area due to their similarities to humans and the availability of genetic and molecular tools. The excisional wound model is widely used for studying wound healing in rats, which typically involves re-epithelialization, granulation tissue formation, and scar formation. Factors such as genetics, age, sex, and overall health status can influence wound healing outcomes. Studies have also explored the role of growth factors and cytokines in wound healing, such as VEGF and IL-6. This model is valuable for studying complex biological processes and evaluating new therapeutic interventions.

7. CONCLUSION:

The study developed a biomaterial using collagen and hyaluronic acid for wound healing in diabetic rats induced by streptozotocin. The biomaterial significantly reduced pro-inflammatory cytokine expression in the wound bed, improved healing, and increased collagen deposition. This suggests potential for developing new treatments for diabetic wounds in humans.

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