



Research Article

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INVESTIGATING THE ROLE OF SESAMOL IN PROMOTING THE HEALING OF DIABETIC WOUNDS BY ANALYZING MOLECULAR EXPRESSION PATTERNS IN HUMAN DIABETIC DERMAL FIBROBLASTS

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SRB assay, RT-PCR

ABSTRACT

Background: Sesamol (3,4-methylenedioxyphenol) is one of the plant compounds tested *in vivo* for diabetic wound healing, normal wound healing, and dexamethasone-induced delayed wound healing. Elucidation of mechanisms underlying the wound healing effect of sesamol through modulation of various molecular and cellular pathways is the crux of this paper. **Objectives:** The objective of the current work was to uncover the mechanism of sesamol underlying the treatment of diabetic wounds using gene expression analysis. **Methods:** The cytotoxicity assay was performed using an SRB colorimetric assay, from which two doses were selected for further studies. The expression of various molecular markers was performed using RT-PCR. **Results:** An SRB assay was carried out to identify the safe concentration of molecules in HDDF cell lines. Two doses that showed more than 80 % viability were selected and used for gene expression analysis. It was observed that sesamol enhanced the expression of VEGF, TGFβ, AKT, JNK, ERK, and TIMP3 significantly ($P \leq 0.001$, $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.0001$) when compared to control and significantly ($P \leq 0.0001$) downregulated the expression of MMP2, MMP-9 when compared to control, which promote wound healing in diabetes. The migration studies also showed a significant increase when compared to the control. **Conclusion:** Sesamol (SM) is a promising molecule that can accelerate wound healing in diabetes by modulating different markers involved in the process.

INTRODUCTION

The major characteristic feature of diabetes mellitus is the complications resulting from it which can cause irreversible changes [1]. The World Health Organisation estimates that 422

million people worldwide have diabetes, and that the disease causes 1.5 million deaths annually [2]. Diabetic foot ulcers are a severe consequence of diabetes mellitus that, if left untreated,

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can result in amputation of the lower limb. Patients' quality of life is negatively impacted by diabetic foot ulcers. Approximately 50% of hospitalizations due to diabetes are attributed to it [3]. Patients with diabetes have a 25% chance of developing foot ulcers, and ulcers can lead to 85% of lower limb amputations. According to reports, the death rate from ulcers varies from 24.6% within 5 years to 45.4% within 10 years [4]. Diabetes-related dysregulated metabolic pathways cause impairments in all phases of wound healing, including delayed wound healing.

All phases of wound healing, hemostasis, inflammation, proliferation, and remodeling are impacted by diabetes, which slows down the healing process. The chronic inflammatory phase, decreased granulation tissue development and angiogenesis, and decreased wound tensile strength are characteristics of diabetic wound healing [5]. Diabetes causes delayed wound healing through a variety of pathways. Hyperglycaemic conditions in diabetes activate the hexosamine pathway, which reduces glucose-6-phosphate dehydrogenase, thereby decreasing NADPH and decreasing myoinositol concentration. Activation of aldose reductase and sorbitol dehydrogenases by hyperglycaemic conditions results in decreased sorbitol or fructose concentration, which also can decrease myoinositol concentration. This reduces impulse transmission and results in neuropathy. Neuropathy reduces cell chemotaxis, growth factor production, and cell proliferation, which all result in delayed wound healing. Hyperglycaemia also reduces nitric oxide synthase, decreasing nitric oxide and increasing reactive oxygen species, affecting vasoconstriction, platelet function, angiogenesis, and inflammation, resulting in delayed wound healing [5].

In diabetic wound healing, as opposed to normal wound healing, there are more macrophages at the wound site, which causes a protracted inflammatory phase [6]. Interleukins are elevated, and growth factor release is decreased. Reduction in growth factor release raises cell apoptosis and decreases fibroblast migration and proliferation. Vascular endothelial growth factor (VEGF) reduction hinders angiogenesis and prevents endothelial progenitor cell immobilization via activating endothelial nitric oxide synthase. Increased concentrations of matrix metalloproteinases (MMPs) and reduced levels of tissue inhibitors of matrix metalloproteinases (TIMPs) impede the growth of the extracellular matrix (ECM). There is less

fibroblast differentiation into myofibroblasts. Reduced chemokines and keratinocyte migration and proliferation hinder angiogenesis, delaying the healing of wounds.

A multidisciplinary approach is introduced in the treatment of DFU. The course of treatment includes revascularization, accommodating orthotics, mechanical off-loading, antibiotic therapy for infections, wound base management, and surgical and mechanical debridement [7]. It needs to be established even if several compositions of sophisticated wound therapies have been produced. The following therapies are in development: platelet-derived growth factors, platelet-rich plasma, keratinocyte-based therapies, biological dressings, Ozone oxygen therapy, hyperbaric oxygen therapy, electromagnetic therapy, and negative pressure wound therapy. All these treatments are expensive and not available to everyone. Plant products have been explored for their role in improving diabetic wounds, which include curcumin, sesamol, naringin, syzgium curcumin, resveratrol, and dehydrozingerone [8]. Sesamol (3,4-methylenedioxyphenol) is one of the plant compounds tested *in vivo* for diabetic wound healing and normal and dexamethasone-induced delayed wound healing [9]. It is obtained from sesame seeds and possesses various actions such as hypolipidemic, wound healing, diabetic wound healing, and prevent oxidative stress. Sesamol's wide spectrum of antioxidant activity across various doses and test systems, including H₂O₂ assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, nitric oxide (NO) scavenging, and lipid peroxidation in the brain and liver has been conducted [10] and in studies conducted on rats, sesamol (SM) exhibited a significant cardioprotective effect against doxorubicin (DOX)-induced cardiomyopathy. Isoproterenol (ISO)-induced cardiotoxicity, associated with increased circulatory lipids, particularly plasma triglyceride (TG) and free fatty acid (FFA) concentrations, was significantly mitigated by SM treatment. Sesamol administration to H9c2 cardiomyoblasts exposed to doxorubicin (DOX) reversed cytotoxic and genotoxic effects induced by DOX, including elevated intracellular reactive oxygen species (ROS) levels, decreased cell viability, and increased apoptosis [11]. In studies involving Huntington's disease and menopause-associated cognitive and emotional disturbances, sesamol demonstrated potential neuroprotective effects. Sesamol-loaded solid lipid nanoparticles (S-SLNs) exhibited neuroprotective effects in a model of cognitive deficit induced by intracerebroventricular streptozotocin (ICV-STZ) administration [12]. Sesamol demonstrated anticonvulsant

activity in a model of pentylenetetrazol-induced seizures, protecting against seizure-induced cognitive impairment and oxidative stress[13]. Using SM-loaded transfersomal gel demonstrated enhanced skin penetration and increased deposition within the skin. Wound healing studies indicated that the highest degree of wound contraction occurred with SL-loaded transfersomes. After 21 days of application, significant improvements in skin histological architecture were evident.

The optimized SM-PLGA nanosuspension exhibited an average particle size below 300 nm, with a PDI < 0.200 and spherical particle morphology. Around 80% of the drug was released over 60 hours in vitro, with a calculated half-life of 13.947 ± 0.596 h. Treatment with SM-PLGA nanosuspension led to decreased levels of TNF- α in wound tissue and accelerated collagen deposition. Moreover, the expression of HSP-27, ERK, PDGF-B, and VEGF was upregulated, promoting the development of

new blood vessels. Rapid re-epithelization, fibroblast migration, collagen deposition, and reduced inflammatory cell infiltration at the wound site were also observed [14]. The composite nanofiber membrane containing a high dose of sesamol (5% of total polymer concentration, w/w) facilitated the formation of myofibroblasts by enhancing TGF- β signaling pathway transduction [15]. It also promoted keratinocyte growth by inhibiting chronic inflammation in wounds, thus enhancing wound healing in diabetic mice.

It is relevant to elucidate the mechanisms behind sesamol's enhancement of delayed wound healing through the modulation of different pathways involved in diabetic wounds. The objective of the current work was to uncover the mechanism of sesamol underlying the treatment of diabetic wounds using expression studies. The hypothetical pathway through which modulation of the wound healing process in diabetes occurs is depicted in Figure 1.

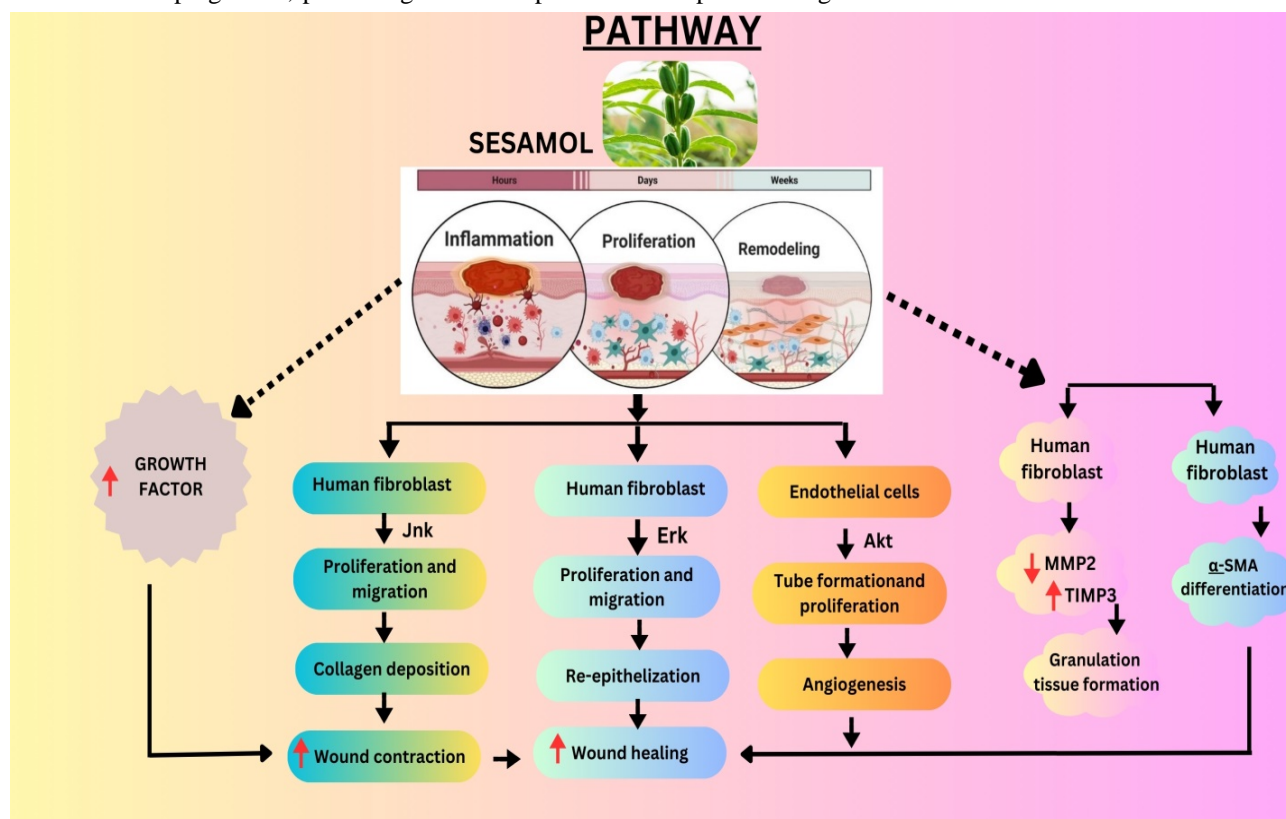


Figure 1: Pathway showing how sesamol modulate the wound healing in diabetes

Based on this pathway we aimed our study to evaluate cell viability of sesamol by SRB assay and evaluation of expression of various molecular markers by RT-PCR. The hypothesis of the present study can be explained. Sesamol influences the stages of delayed diabetic wound healing, including inflammation, proliferation, and remodeling. During inflammation, it boosts

the expression of growth factors, leading to enhanced wound contraction and improved healing in diabetes. In the proliferation phase, sesamol enhances the proliferation and migration of human dermal fibroblasts by increasing JNK and ERK expression. It also promotes collagen deposition and reepithelialization, accelerating wound healing in diabetic

conditions. In the remodeling phase, sesamol reduces MMP2 and MMP9 levels while enhancing TIMP3, facilitating granulation tissue formation and wound healing. Sesamol promotes α SMA differentiation, increases wound contraction, and improves delayed wound healing.

MATERIALS AND METHODS

***In vitro* cytotoxicity effect of sesamol determination by sulforhodamine b(SRB) colorimetric assay**

The SRB assay determines the safe doses at which the study can be conducted. Human diabetic dermal fibroblasts were initially procured from Zen Bio, USA, and maintained in a dermal fibroblast growth medium and a fibroblast growth factor supplement (Zen Bio). The cell line was cultured in a 25 cm² tissue culture flask using Dermal fibroblast growth medium supplemented with fibroblast growth factor and an antibiotic solution consisting of Penicillin (10U/mL), Streptomycin (10 μ g/mL), and Amphotericin B (25 ng/mL). Once the cell line reached sufficient confluency, the cells were detached using 500 μ l of 0.025% Trypsin in PBS/0.5mM EDTA solution (Himedia), neutralized with Trypsin neutralizing solution, centrifuged at 125xg for 10 minutes, and resuspended in 10% growth medium. A 100 μ l cell suspension containing 5x10³ cells per well was then seeded into a 96-well tissue culture plate and placed in a humidified 5% CO₂ incubator at 37°C for incubation.

Preparation of stock solution:

1 milligram of the sample was weighed and dissolved in 1 milliliter of medium using a cyclomixer. To ensure sterility, the resulting solution was filtered through a 0.22 μ m Millipore syringe filter.

Cytotoxicity Assessment:

After 24 hours, the cells were exposed to samples of varying concentrations (100 μ g, 50 μ g, 25 μ g, 12.5 μ g, 6.25 μ g in 500 μ l of medium) and incubated for an additional 24 hours before commencing the staining procedure.

Sulforhodamine B (SRB) Staining

Fixation and Staining of Cells

In each well of the plate, 100 μ l of cold 10% TCA was added without removing the cell culture supernatant and then incubated at 4°C for 1 hour. Afterward, the plates were washed four times with gently flowing tap water, and excess water was removed by blotting with paper towels. The plates were then dried

completely using a blow dryer. Once dry, 100 μ l of 0.057% SRB solution was added to each well and incubated for 30 minutes. Following this; the plates were briefly rinsed four times with 1% acetic acid to remove any excess unbound dye. Subsequently, 200 μ l of 10mM Tris base solution (pH 10.5) was added to each well, and the plates were agitated on a shaker for 5 minutes to dissolve the protein-bound dye. Optical density (OD) measurements were taken using a microplate reader at 510nm. Throughout these procedures, the plates were kept at 37°C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany) [16].

$$\% \text{ Cell Viability} = \frac{\text{Mean OD samples}}{\text{Mean OD of Control group}} \times 100$$

Effect of sesamol on cell migration in human diabetic dermal fibroblasts cells by Transwell migration assay

Initially obtained from Zen Bio, USA, human diabetic dermal fibroblasts were sustained in Dermal Fibroblast Growth Medium supplemented with fibroblast growth factor. Cultured in 25 cm² tissue culture flasks with the same medium alongside an antibiotic solution (Penicillin, Streptomycin, and Amphotericin B), these cell lines were maintained in a humidified 5% CO₂ incubator at 37°C. The transwell migration assay entails the directional movement of cells through a porous membrane, facilitated by a chemoattractant gradient established between two compartments filled with medium [17].

The bottom chamber is filled with a medium containing the chemoattractant, while cells are seeded in the top chamber, establishing a gradient conducive to cellular migration. Following incubation, the extent of cell migration can be evaluated by quantifying the adherent cells migrating to the porous membrane's basal side. Procedure: Exponentially growing cells were trypsinized and seeded at a density of 200,000 cells per well in a 12-well plate for a 24-hour incubation period (approximately 90% confluence). The cells were then treated with samples of varying concentrations (6.25 μ g/mL and 25 μ g/mL). Non-treated control cells were also maintained and incubated for 24 hours. After this incubation period, cells were detached from the tissue culture plate using a 0.25% trypsin-EDTA solution, centrifuged, and the existing media were aspirated, leaving behind the pelleted cells. Cells were then resuspended in serum-free cell culture media. Subsequently, 100 μ L of cell solution was plated on top of the filter membrane in a transwell insert and incubated for 10 minutes at 37°C with 5% CO₂ to allow the cells to settle. Following this, 600 μ L of

DMEM supplemented with 10% FBS was carefully added to the bottom of the lower chamber in a 24-well plate using a pipette. Cultures were then incubated for an additional 24 hours. After 24 hours, the transwell insert was removed from the plate, and a cotton-tipped applicator was used to carefully remove the media and remaining cells from the top of the membrane without causing damage. The transwell insert was then placed in a well of a 24-well plate containing 70% ethanol for 10 minutes to fix the cells. Following fixation, the insert was removed from the ethanol, excess ethanol was removed from the membrane using a cotton-tipped applicator, and the membrane was allowed to dry for 10-15 minutes. Subsequently, the membrane was stained with 0.2% crystal violet solution in a 24-well plate at room temperature for 5-10 minutes. After staining, excess crystal violet was removed from the membrane, and the membrane was allowed to dry. Cells that migrated through the membrane and adhered to the underside were counted under an inverted microscope in different fields of view to determine the average number of migrated cells.

Effect of sesamol on mRNA expression in human diabetic dermal fibroblast cells by Real time PCR analysis

For RT-PCR, cells were cultured following standard procedures. After 24 hours, the cells were treated with varying sample concentrations (6.25µg/mL and 25µg/mL). Control cells were also maintained and incubated for 24 hours.

TRIzol Method for isolation of RNA

Total RNA (TRIzol Method) was isolated using a total RNA isolation kit according to the manufacturer's instructions (Invitrogen—Product code 10296010). The addition of TRIzol solution disrupted the cells, releasing RNA. During chloroform extraction and subsequent centrifugation, RNA separated into the aqueous phase, while proteins were located in the interphase and organic phase. Upon mixing with isopropanol, RNA precipitated as a white pellet at the side and bottom of the tube. To initiate the process, 1ml of TRIzol reagent was added to the culture well plate and incubated for 5 minutes. The contents were then transferred to a fresh sterile Eppendorf tube. Subsequently, 200µL of chloroform was added at room temperature, followed by centrifugation at 14000rpm for 15 minutes at 40°C. The resulting aqueous layer was collected, and 500µL of 100% isopropanol was added. After incubating for 10 minutes at room temperature, the mixture was centrifuged at 14000rpm for 15 minutes at 40°C. The supernatant was

discarded, and the pellet obtained was washed with 200µL of 75% ethanol (Merck). It was then centrifuged at 14000rpm for 5 minutes at 40°C in a cooling centrifuge (Remi CM12). The RNA pellet was dried and suspended in TE buffer [18].

cDNA Synthesis

Total RNA was isolated using Trizol (Invitrogen, USA), and subsequent assessment determined its purity and concentration. Complementary DNA (cDNA) was synthesized using the cDNA preparation kit (G BIOSCIENCES, Product code- 786-5019s,786-5020, master premix for first-strand cDNA synthesis). In an RNase free tube, a mixture containing 5µl of RT Easy mix, 0.5µl of oligodT, and 2 µl of RNA template (equivalent to 0.5µg of total RNA) was prepared. Sterile distilled water was added to achieve a total reaction volume of 10 µl, followed by gentle pipetting to mix the solution thoroughly. The cDNA synthesis was carried out using a thermal cycler (Eppendorf Master Cycler) programmed for specific conditions, involving 20 minutes at 42°C followed by 5 minutes at 85°C.

Table 1: Cycling conditions

Step	Temperature °C	Time (minutes)	Number of cycles
cDNA synthesis	42	20	1
Inactivation	85	5	1

Gene expression analysis via RT-qPCR

Real-time qRT-PCR analysis was conducted using SYBR Green Master Mix (G BIOSCIENCES, Product code-786-5062) on a Lightcycler 96 (Roche). Each reaction was replicated three times, and data were assessed using the $\Delta\Delta C_t$ method (utilizing Light Cycler 96 SW 1.1 Software). Table 2 provides information on the different steps, durations, and temperatures.

Table 2: outlines the steps involved, the duration of each step, and the corresponding temperatures

Steps	Time required	Temperature
Initial activation step	2 minutes	95°C
3 step cycling		
Denaturation	10 seconds	95°C
Annealing	1 minute	58°C
Extension	1 min/kb	72°C
Number of cycles	40 cycles	68°C
End of PCR cycling	Indefinite	4°C

The primer sequences utilized are summarized in Table 3.

Table 3: Primer sequence

OLIGO NAME	FORWARD		REVERSE	
	SEQUENCE (5' ->3')	Tm	SEQUENCE (5' ->3')	Tm
H-GAPDH	ACTCAGAAGACTGTGGATGG	57.3	GTCATCATACTTGGCAGGTT	55.3
TGF β	TGGAGCAACATGTGGAAGCTC	57.3	TGCCGTACAACCTCCAGGTGAC	59.4
H- α SMA	CTATGCCTCTGGACGCACAACT	62.1	CAGATCCAGACGCATGATGGCA	62.1
MMP2	CAACTACAACCTTCTTCCCTCGCA	60.7	GGTCACATCGCTCCAGACTTG	61.8
TIMP3	TACCGAGGCTTACCAAGATGC	62.1	CATCTTGCCATCATAGACGCGAC	62.1
VEGF1	GACCCTGGTGGACATCTTCCAGGA	66.1	GGTGAGAGGTCTAGTTCCCGA	61.8
AKT	TCTATGGCGCTGAGATTGTG	57.3	CTTAATGTGCCCGTCCTTGT	57.3
H ERK1	TGGCAAGCACTACCTGGATCAG	62.4	GCAGAGACTGTAGGTAGTTTCCGG	62.4
H-JNK 1	GACGCCTTATGTAGTGACTCGC	62.1	TCCTGGAAAGAGGATTTTGTGGC	60.6
MMP9	GCGGAGATTGGGAACCAGCTGTA	64.2	GACGCGCCTGTGTACACCCACA	65.8

Agarose gel electrophoresis separates and observes DNA fragments based on their size and charge. DNA fragments move through an agarose gel matrix in this technique when subjected to an electric field. The electric field is generated by applying a potential across an electrolyte solution (buffer). Agarose, when dissolved in an aqueous buffer by boiling, solidifies upon cooling to form a gel. A 1.5% agarose gel was prepared in 1x TE buffer and melted in a hot water bath at 90°C. After melting, the agarose was cooled to 45°C. Ethidium bromide (6 μ l of 10 mg/mL) was added to the melted agarose, which was then poured into a gel casting apparatus along with a gel comb. The comb was removed once the gel was set, and the electrophoresis buffer was poured into the gel tank. The platform holding the gel was placed in the tank to submerge the gel in the buffer. Samples were loaded onto the gel, and electrophoresis was conducted at 50 V for 30 minutes. The stained gel was observed using a gel documentation system (E gel imager, Invitrogen) [19].

RESULTS

The study was performed on human diabetic dermal fibroblasts cultured in a fibroblast growth medium with additional supplements. SRB assay was performed first to find the safer concentrations for the study. The IC₅₀ value was found to be 156.1489 μ g/mL (Calculated using ED50 PLUS V1.0 Software). Two doses (25 μ g/ml and 6.25 μ g/ml) of sesamol, which showed more than 80% cell viability in HDDF cell lines, were selected. Migration studies showed significant enhancement in the migration of cells in test groups when compared to control. Further, molecular expression studies using both concentrations were carried out in cell lines. In expression studies, SM treated group showed upregulation of VEGF, TGF β , AKT, JNK, ERK,

and TIMP3 significantly compared to control and significant downregulation of MMP2 compared to control, which promotes wound healing in diabetes. The characteristic features of diabetic wounds are a reduction in growth factors and a reduction in the expression of cell fate regulators. Also, in diabetic wounds, an increase in MMP2 expression and a decline in TIMP3 expression are observed. In the present study, sesamol enhanced the expression of growth factors and cell fate regulators. Also, the compound has reduced the expression of MMP2 and increased the expression of TIMP3. Migration studies showed significant enhancement in the migration of cells in test groups when compared to the control.

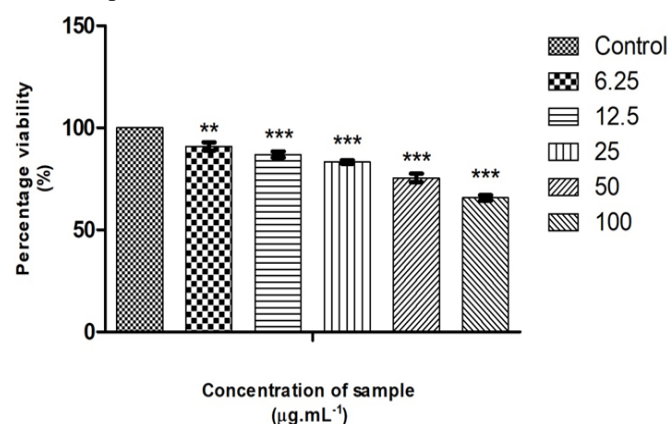


Figure 2: Graphical representation depicting the cytotoxic effect of a sample on HDF cells by SRB assay. There is a varied sample concentration along the Y-axis, Percentage viability, and the X-axis. All experiments were done in triplicates, representing the results as Mean \pm SE. One-way ANOVA and Dunnett's test were performed to analyze data. ***p < 0.001 compared to control groups, **p < 0.01 compared to control groups.

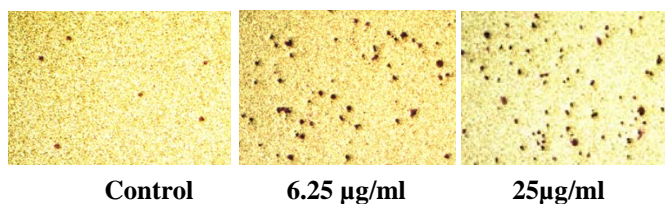


Figure 3: Effect of sesamol on migration of cells

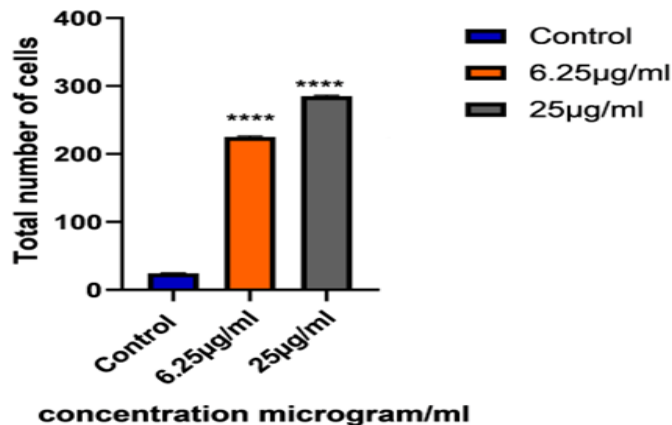


Figure 4: Effect of sesamol in migration of cells. Along the X axis is the sample concentration, and along the Y axis is the total number of cells. Datas are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test. ****P ≤ 0.0001 when compared to control

The impact of SM on the mRNA expression of growth factors in HDDF cell lines.

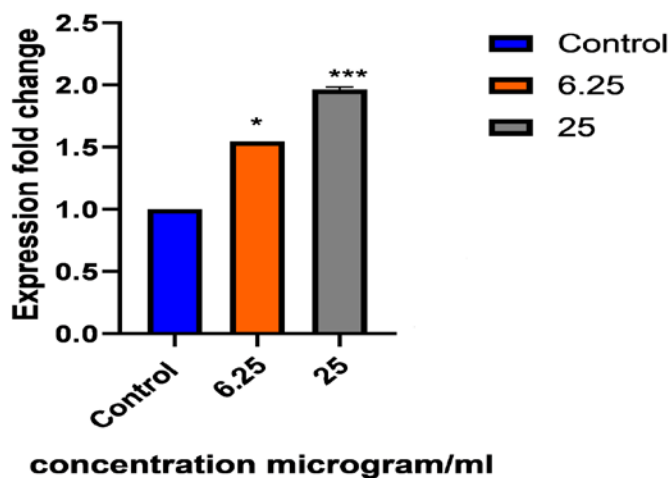


Figure 5: Effect of sesamol on mRNA expression of VEGEF. Along the X axis is the sample concentration, and along the Y axis is the Expression fold change. Datas are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test. ***P ≤ 0.001, *P ≤ 0.05 when compared to control
 ***P ≤ 0.001 * P ≤ 0.05 when compared to control

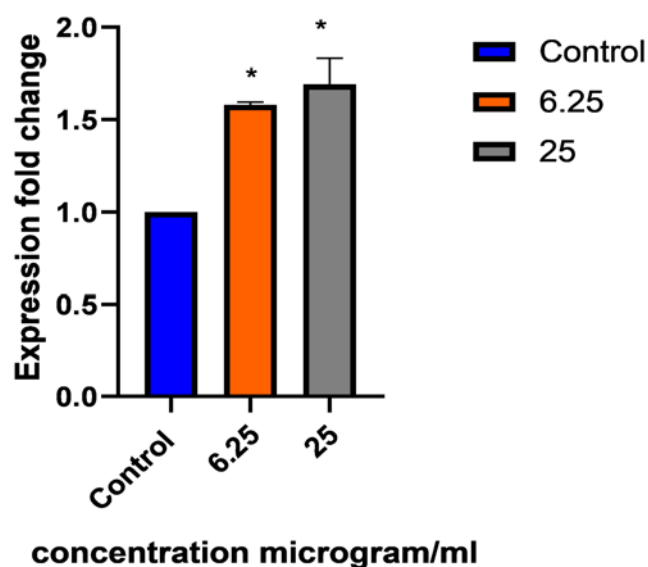


Figure 6: Effect of sesamol on mRNA expression of TGF β. Along the X axis, the concentration of the sample, and the Y axis, the Expression fold changes. Datas are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test. * P ≤ 0.05 when compared to control

The influence of SM on mRNA expression of cell fate regulators in HDDF cell lines.

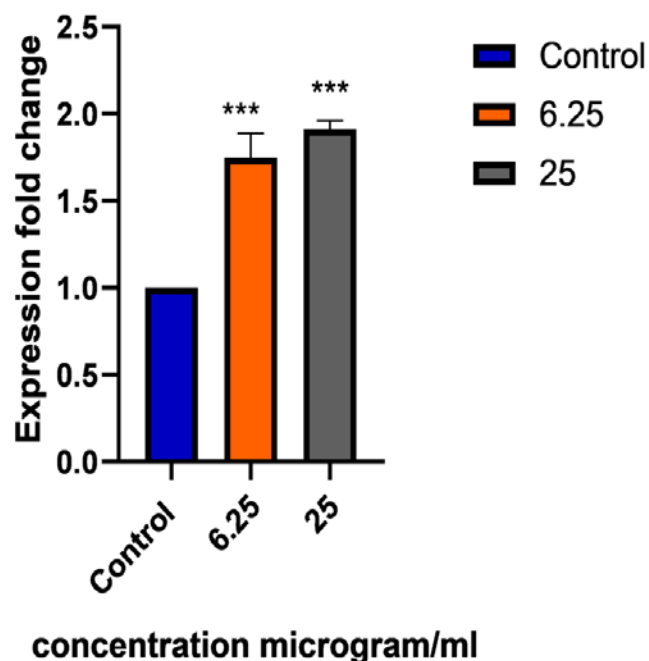
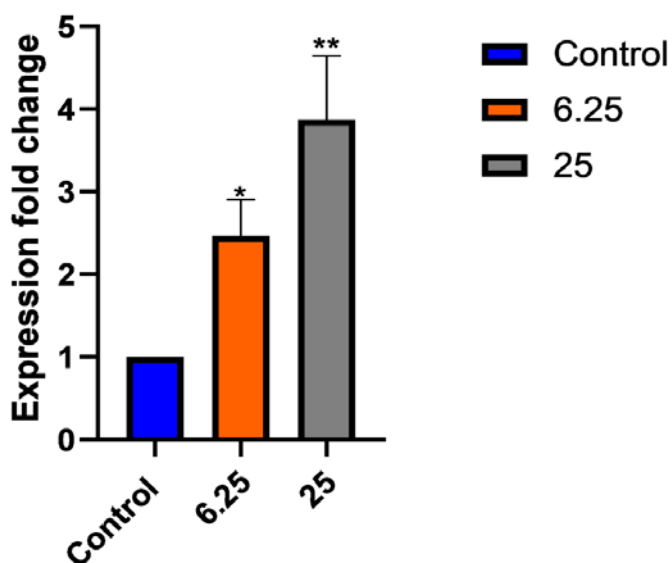


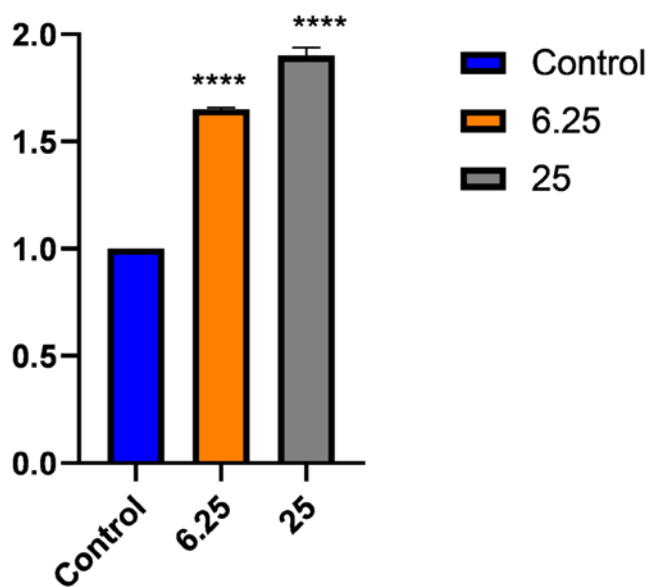
Figure 7: Effect of sesamol on mRNA expression of AKT. Along the X axis is the concentration of sample and along the Y axis is the Expression fold change. Datas are expressed as Mean ±SEM. One way ANOVA is performed followed by Tukey’s multiple comparison test
 ***P ≤ 0.001 when compared to control



concentration microgram/ml

Figure 8: Effect of sesamol on mRNA expression of JNK. Along the X axis is the sample concentration, and the Expression fold changes along the Y axis. Data are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test

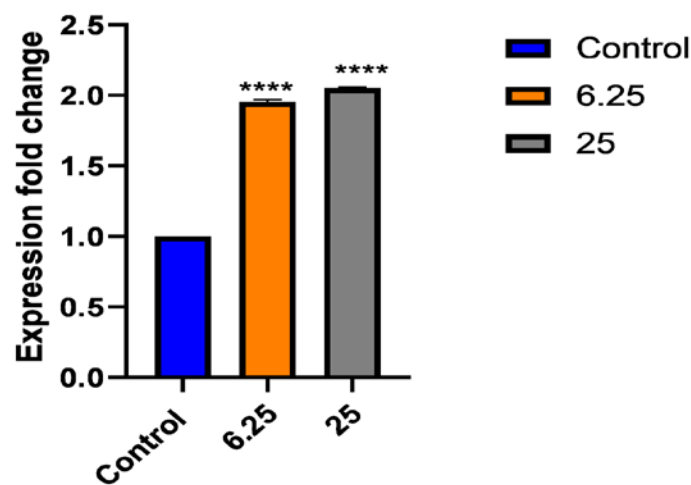
*P ≤ 0.05 **P ≤ 0.01 when compared to control



concentration microgram/ml

Figure 9: Effect of sesamol on mRNA expression of ERK. Along the X axis is the concentration of sample and along the Y axis is the Expression fold change. Data are expressed as Mean ±SEM. One way ANOVA is performed followed by Tukey’s multiple comparison test. ****P ≤ 0.0001 when compared to control

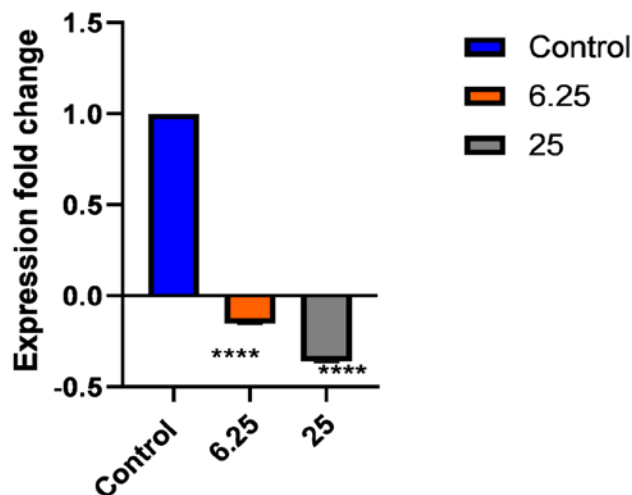
The impact of SM on the mRNA expression of α SMA in HDDF cell lines.



concentration microgram/ml

Figure 10: Effect of sesamol on mRNA expression of α SMA. Along the X axis, the concentration of the sample, and the Y axis, the Expression fold changes. Datas are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test. ****P ≤ 0.0001 when compared to control

The effect of sesamol in mRNA expression of MMP2, MMP9, TIMP3



concentration microgram/ml

Figure 11: Effect of sesamol on mRNA expression of MMP2. Along X axis concentration of sample and along Y axis Expression fold change. Datas are expressed as Mean ±SEM. One way ANOVA is performed followed by Tukey’s multiple comparison test. ****P ≤ 0.0001 when compared to control

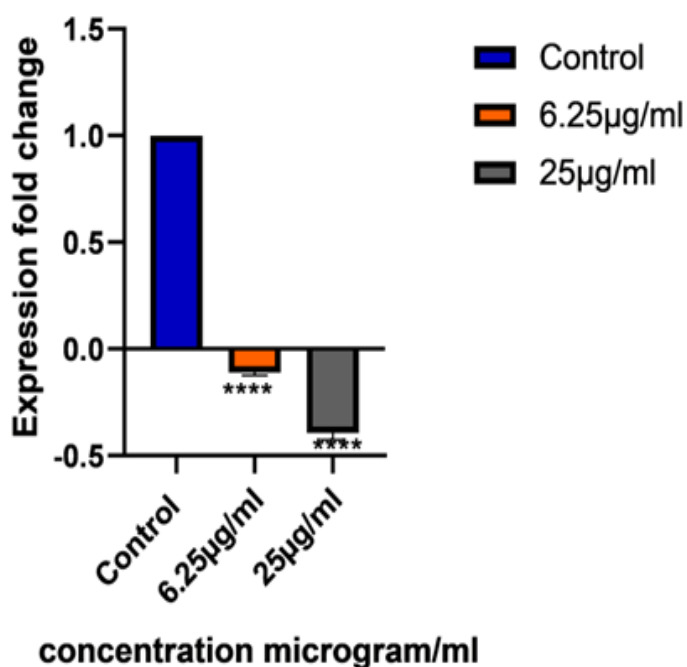


Figure 12: Effect of sesamol on mRNA expression of MMP9. Along the X axis, the concentration of the sample, and the Y axis, the Expression fold changes. Datas are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test
 ****P ≤ 0.0001 when compared to control

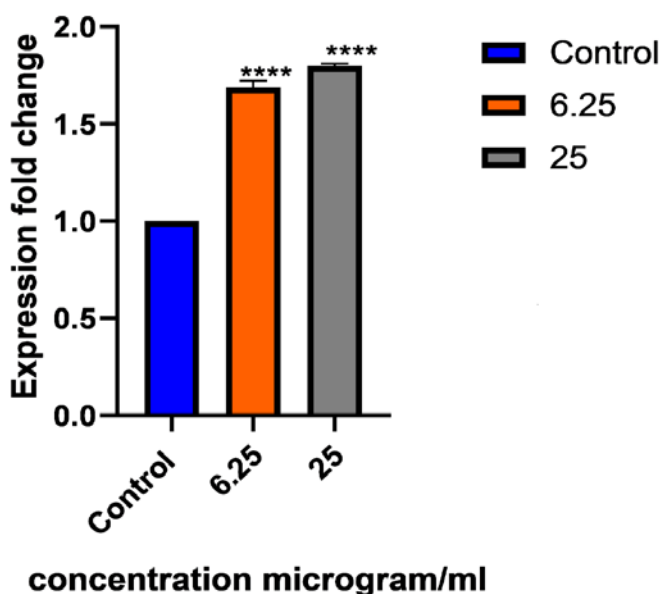


Figure 13: Effect of sesamol on mRNA expression of TIMP3. Along the X axis, the concentration of the sample, and the Y axis, the Expression fold changes. Datas are expressed as Mean ±SEM. One-way ANOVA is performed, followed by Tukey’s multiple comparison test N. ****P ≤ 0.0001 when compared to control

Agarose gel electrophoresis

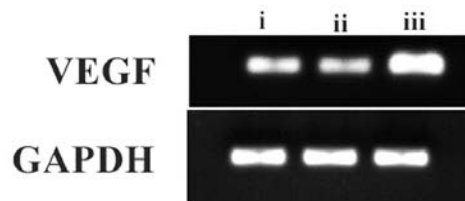


Figure 14: Agarose gel electrophoresis of VEGF when compared with GAPDH, where i. Control, ii. Test (6.25 µg/ml), iii. Test (25 µg/ml)

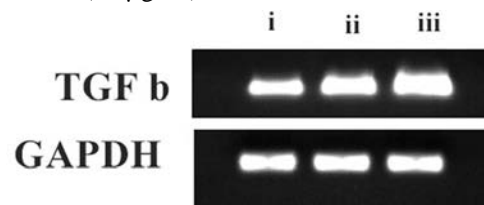


Figure 15: Agarose gel electrophoresis of TGF β when compared with GAPDH, where i. Control, ii. Test (6.25 µg/ml), iii. Test (25 µg/ml)

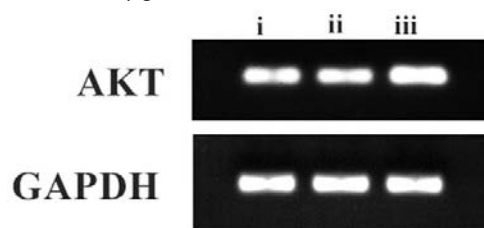


Figure 16: Agarose gel electrophoresis of AKT when compared with GAPDH, where i. Control, ii. Test (6.25 µg/ml), iii. Test (25 µg/ml)

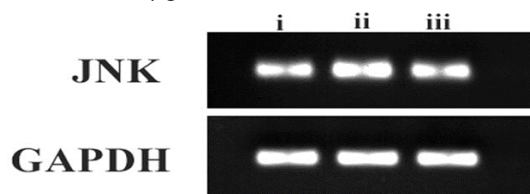


Figure 17: Agarose gel electrophoresis of JNK when compared with GAPDH, where i. Control, ii. Test (6.25 µg/ml), iii. Test (25 µg/ml)

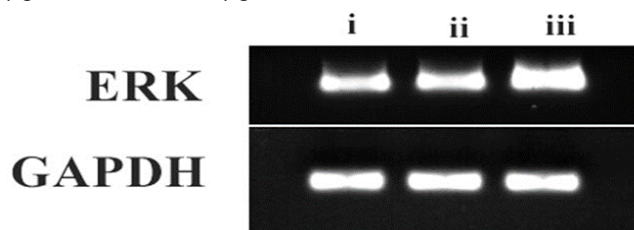


Figure 18: Agarose gel electrophoresis of ERK when compared with GAPDH, where i. Control, ii. Test (6.25 µg/ml), iii. Test (25 µg/ml)

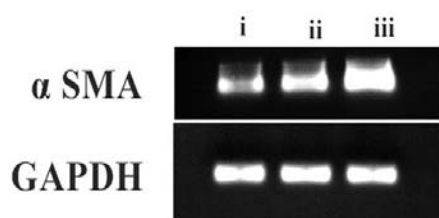


Figure 19: Agarose gel electrophoresis of α SMA when compared with GAPDH, where i. Control, ii. Test (6.25 μ g/ml), iii. Test (25 μ g/ml)

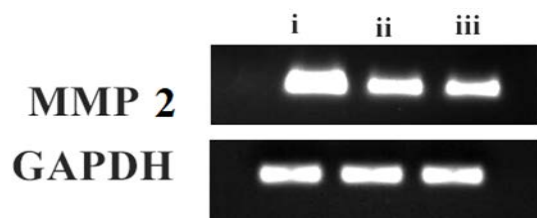


Figure 20: Agarose gel electrophoresis of MMP2 compared with GAPDH, where i. Control, ii. Test (6.25 μ g/ml), iii. Test (25 μ g/ml)

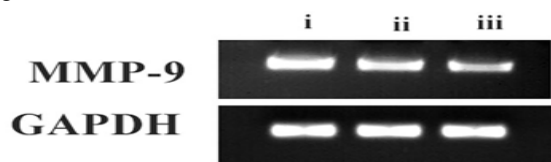


Figure 21: Agarose gel electrophoresis of MMP9 compared with GAPDH, where i. Control, ii. Test (6.25 μ g/ml), iii. Test (25 μ g/ml)

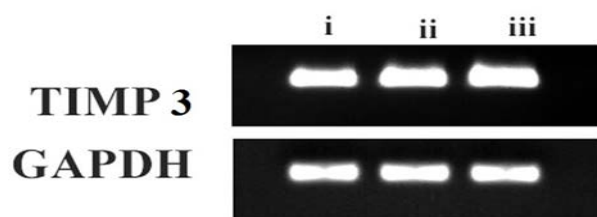


Figure 22: Agarose gel electrophoresis of TIMP3 when compared with GAPDH, where i. Control, ii. Test (6.25 μ g/ml), iii. Test (25 μ g/ml)

DISCUSSION

Delayed wound healing is one of the characteristic features of diabetes which occurs by altering each phase in wound healing. Debridement, dressings, transcutaneous electrical nerve stimulation, nanomedicine, hyperbaric oxygen therapy, photobiomodulation, and shock wave therapy are some of the conventional methods currently used to treat diabetic wounds. However, these methods have limitations when it comes to

regulating wound healing, and the likelihood of a recurrence is also higher[20]. Sesamol (SM) a strong antioxidant present in sesame seeds has role in regulating wound healing[21]. In the present study we analysed the molecular mechanisms behind the wound healing potential of sesamol in chronic wounds such as diabetic wounds.

The present study focused on evaluating the role of SM in diabetic wounds mechanistically by modulating different markers involved in the process of delayed diabetic wound healing. Different pathways including these analysed markers modulate the wound healing process. VEGF and TGF β pathway enhances angiogenesis and promotes wound closure. TGF β induces epithelial mesenchymal transition thus enhances the wound healing process. AKT signalling pathway, activation has observed to increase impaired wound healing. Dysfunction of the pathway is observed in diabetic mice. The wound healing was promoted by the activation of JNK signalling pathway JNK pathway regulates production of proinflammatory cytokines, chemokines, MMPs. Acceleration of ERK pathway enhances the wound healing in diabetes. Balance between MMPs and TIMP3 modulates the healing process in diabetes. Here, in the study we evaluated the effect of SM (two doses selected by SRB assay) in modulating expression of VEGF and TGF β . The characteristic feature of diabetic wounds is the reduction in growth factors such as VEGF, TGF β and rapid proteolysis of same. Our study showed that SM increased the expression of VEGF, TGF β significantly when compare to control. Next we evaluated the expression of cell fate regulators; interestingly SM enhanced the expression of AKT, JNK, and ERK significantly. Diabetic wounds are characterised by reduction in cell fate regulators. Studies have shown that α SMA, a well-accepted marker of myofibroblast differentiation have role in the production of contractile force during wound healing, the expression of which is reduced in diabetic studies. From the study it has been observed that SM significantly enhanced α SMA expression when compare to control. Studies suggested that patients with non-healing chronic ulcer showed overexpression of MMP-2, MMP-9 and reduced TIMP3 expression. As results the balance between ECM and degradation enzymes are altered in delayed diabetic wounds. It was observed in the present study that SM decreases the expression of MMP-2, MMP-9 and enhanced the expression of TIMP3 significantly when compare to control. Thus, by modulating different molecular markers involved in the delayed

diabetic wound healing process of diabetes, SM accelerates the healing process mechanistically. The migration study showed a significant increase in the migration of cells at the selected dose. The characteristic feature of delayed diabetic wounds is the impairment in the migration of cells that delays the healing process.

CONCLUSION

Sesamol(SM) is a promising molecule that can accelerate wound healing in diabetes by modulating different markers involved. By analyzing the expression behind various important markers, the study proved the mechanistic effect of sesamol in accelerating diabetic wounds.

Future Perspectives

Sesamol formulation can be scaled up for which preclinical and clinical studies need to be performed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

The conception of the work was done by Fathima Beegum and Rekha R Shenoy. The work was designed by Fathima Beegum and Rekha R Shenoy. The work was performed, and analyzed, and an interpretation of results was made by Fathima Beegum. Draft preparation was done by Fathima Beegum. The draft was edited by Rekha R Shenoy. Design of work, edited draft and revised was done by Nandakumar Krishnadas. Anuranjana P V, Krupa Thankam George, Divya K P and Farmiza Begum did Draft preparation

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