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Benzylacetophenones ring containing Chalcones deciphers augmented cell migration by Stabilizing basement Matrix in mouse embryonic fibroblast

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Abstract

Background:

As a result of harmful stimuli, the body's plasma and immune cells are drained from the rest of the body and directed toward the injured tissue. To encourage the regeneration of damaged tissue, a signaling cascade involving growth factors and cytokines propagates and matures in the inflammatory site. This cascade involves blood vessels and immune markers in the injured tissue.

Objective:

To evaluate the anti-inflammatory, wound healing and antioxidant properties of Phloretin in LPS stimulated L929 Fibroblast cell.

Methods:

L929 Fibroblast cells were used to study the wound healing efficiency of Phloretin. Wound healing efficiency of Phloretin was investigated by scratch assay. Phloretin was studied to see its ability to reduce nitric oxide production by Griess Assay, tumor necrosis factor – α expression in L929 Fibroblast were analyzed by ELISA. Phloretin's effect on ECM Components analysis was done to check the content of Hydroxyproline and Hexosamine.

Results:

Phloretin reduces NO generation in LPS-exposed fibroblasts. Phloretin inhibited LPS-activated L929 fibroblast TNF-α production. LPS-treated phloretin had pronounced expression of hydroxyproline, indicating accelerated cell proliferation, collagen formation, and fast healing. The stability of hexosamine was also stabilized in comparison with the control.The recolonization and migration of cells was also augmented by scratch and chemotaxis assay upon treatment with Phloretin.

Conclusion:

In other words, this study suggests that reducing nitric oxide and unsettling the inflammation-causes eventful wound healing which is otherwise prolonged due to disproportionate chemokine and cytokine expression in pathological circumstances.

Keywords: Phloretin, Inducible NOS (iNOS), Matrix-Metalloproteinase, Extracellular matrix, Reactive Oxygen species, L929 Fibroblast.

INTRODUCTION

Wounds are the seeable results of individual cell damage that can be categorized based on size, depth, location, cause such as accident or surgery or circulatory failure. In order for a wound to heal, there must be a continuous flow of events that restore the integrity of the injured tissue: the inflammatory, proliferation, and remodeling stages[1]. Platelets, inflammatory cells, fibroblasts, keratinocytes, as well as cytokines, growth factors, and matrix metalloproteinases (MMP), are all involved in this process[2]. After injury, instantly inflammation stage begins with vascular constriction that seeks to promotes homeostasis while releasing inflammation mediators. The proliferative stage is differentiated by granulation tissue proliferation which is primarily caused by fibroblast as well as the angiogenesis process. Endothelial cells are present in the injured area 2–4 days after wounding, accompanied by fibroblasts. Due to injuries, a variety of substances, such as platelet-derived growth factor and transforming growth factor, attract fibroblasts to the wound. Inside the wound, fibroblasts multiply and start producing the matrix protein molecules. These elements help in the formation of the new extra - cellular matrix, which promotes cell ingrowth and is required for the healing period. Fibroblasts and the extracellular matrix have an important connection that serves to govern extracellular matrix formation and subsequent remodeling [3]. Extracellular matrix acts as a substrate surface for cell adhesion and governs the growth, mobility, and distinction of the cells that reside within it, as well as providing turgidity to soft tissues and stiffness to bone. The extracellular matrix is composed of fibrous structural protein molecules and an intercellular matrix made up of sticky glycoproteins engrained in a proteoglycan and glycosaminoglycan gel[4]. In order for wounds to heal and scars to form, there must first be a breakdown of the extracellular matrix (ECM). MMPs are responsible for the processing of cytokines and growth factors, as well as the hydrolysis of major ECM molecules such as collagen, elastin, laminin, and fibronectin[5]. Hydroxyproline, of a protein component of collagen, is released during the breakdown of the ECM component. Hexosamine, which is a component glycosaminoglycan, is the Matrix molecule that acts as Ground Substance for synthesis of ECM.

Chemotaxis is a very important part of many different physiological processes, such as bringing leukocytes to places where there is an infection, moving lymphocytes around the body, and forming patterns in neuronal cells in the developing nervous system. Chemokines are small, chemotactic cytokines (8–12 kDa) that control how cells move to an injury site. Chemokines are made by many different types of cells in a wound, such as endothelial cells, fibroblasts, keratinocytes, neutrophils, and macrophages. Because chemotaxis is so important for development and physiology, cells that move in the wrong way can lead to a number of diseases, such as tumour growth, cancer metastasis, and inflammatory diseases like asthma, arthritis, and atherosclerosis[6].

The remodeling stage is identified by modifications and developments in the collagen fiber components that enhances the tensile strength[7]. LPS stimulates the regulation pro-inflammatory cytokines like Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1β) and interlukin-6 (IL-

6)[8].It's very well understood that the initiation of these inflammatory mediatorplays a vital role in inflammation mediation and has fatal consequences for the host, ass it is engaged in the process by which arachidonic acid is converted into leukotrienes, thromboxane and prostaglandins via the action of cyclooxygenase (COX) and mass nitric oxide production (NO) [9,10]. NO, a highly reactive substance, which is Synthesized from L-arginine-N-methyl ester (L-NAME) by NO synthase (NOS). Inducible NOS (iNOS) and constitutive NOS are 2 types of NOS. It has been noted that the iNOS is expressed in variety of cells, including smooth muscle cells, bone marrow cells, monocytes and macrophage and it generates large amount of NO when stimulated by pro-inflammatory cytokines [11].

Chronic inflammatory responses enhance mucosal damage leading to pain, swelling, redness and some other symptoms. The production of eicosanoids, prostaglandins, leukotrienes, and reactive oxygen species (ROS) all contribute to the body's ability to heal itself. In particular, reactive oxygen species (ROS) play a significant part in the healing process and act as cellular messengers in a variety of aspects of molecular and cell biology. At the site of a wound, an abundance of reactive oxygen species (ROS) is produced as a defense mechanism against microbial infection. Simultaneously, the presence of free radicals, that could also harm wound in and around cells, or microbial infection could impede the healing process [12].

Phloretin is a natural phenol that is a dihydrochalcone. They are a solid, pearl white powder that smells like sweat. Phloretin is completely soluble in organic solvents like ethanol. They can be found in fibroblasts and platelets. Because clinical studies show that it acts as an antioxidant, it is used in a variety of skin care treatments. They have been shown in clinical studies to have skin-soothing and antioxidative properties. For troubled skin, phloretin can be a helpful soothing ingredient. Due to their polarity, phloretin is thought to have a better transdermal delivery mechanism, allowing it to penetrate the skin more effectively. Phloretin's specific effect in the human body is that it inhibits metastasis in human cervical cancer cells. Phloretin's activity kills microbes, is not allergic, doesn't cause cancer, protects liver and also doesn't cause inflammation.

2. Materials and Methods

2.1. Chemicals and drugs

Dulbecco's modified essential medium (DMSO), dimethyl sulfoxide, fetal bovine serum (FBS), L-nitro-arginine methyl ester (L-NAME) and-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), RIPA Buffer (10X), sulphuric acid, chloroform, liquid ammonium hydroxide was purchased from HiMedia Laboratories(Mumbai, Maharashtra, India). Phloretin, sulfanilamide and naphthyl ethylenediamine were procured from Sigma-Aldrich Chemicals Private Limited (Bangalore, Karnataka, India).

2.2. Cell culture studies

L929 Fibroblast was grown in DMEM supplemented with 10% FBS and 5% antibiotic i.e., 100X Liquid in 10,000 U Penicillin and 10mg Streptomycin per ml in 0.9% normal saline, was acquired from HiMedia in Mumbai, Maharashtra, India) in an atmosphere containing 5% CO_2 [13,14].

2.3. Cell viability assessment of Phloretin in L929 Fibroblast cell line

Reduction of MTT (3- [4, 5-dimethylthiazol- 2-yl]-2, 5-diphenyltetrazolium bromide) was used in checking the cell viability in L929 Fibroblast. Cell seeding was done on a 96- well plate and after the 24 h of incubation, it was then treated with different compounds that were incubated again for another 24 h. After 4hr of incubation ,100µl of MTT solution was added to every well. Later, the medium was aspirated to dissolve the insoluble purple formazan crystals with the solubilizing agent (DMSO). The absorbance was measured at 570nm. The percentage of cell viability was determined by using the formula given below[15]

$$\% \text{ Cell viability} = \frac{O.D\ of\ test}{O.D\ of\ control} * 100$$

2.4. LPS induced nitrite production in L929 Fibroblast cell line.

1 µg/mL of LPS stimulation was used to study the inhibitory effect of Phloretin NO production using L929 Fibroblast cell line. A 96-well plate was taken, and, in the plate, L929 Fibroblast cells were plated. After achieving 80% confluency, 1µg/ml of LPS i.e., Lipopolysaccharide was added to each of the well expect the control.Before the previous step, 10µL of Phloretin and NO synthase inhibitor L-NAME (200µM) was used as positive control was added.For 1 hour, the plate was incubated.After incubating, 1µg/ml of LPS was added to each of the well expect the control.For 24hours, the cells were incubated at 37°C. Following incubation, the supernatant was used to estimate nitrite using the Griess nitrite assay[16].

2.4.1. Griess nitrite assay

An equal amount of supernatant and Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine in 5% phosphoric acid) was mixed thoroughly and incubated at room temperature for 10 minutes. At 540nm, the absorbance was measured[16]. As a control, sodium nitrite was used.

2.5. Efficacy of Cell Migration of Phloretin in L929 Fibroblast cell line

Scratch assay was disbursed for the examination of cell migration using L929 cell line. On the achievement of 80% confluency, a fine space per each well was scratched with a sterile 1 ml micropipette tip to come back up with the same gap that was free from adherent cells. L929s were then treated with different compounds, then again incubated for yet another 24 hours. Employing a phase-contrast microscope, the gap closure was monitored then defined as the mean percentage of the enduring cell-free area after 24 h of treatment, where the initial wound area was compared[17].

$$\% \text{ Cell migration} = \frac{Distance\ within\ Scratch\ (0hrs) - Distance\ within\ Scratch\ (19hrs)}{Distance\ within\ Scratch\ (0hrs)} * 100$$

2.6. Transwell assay

A transwell chemotaxis test in a Boyden chamber was used to measure cell migration. About 2×10^4 cells were suspended in the basal cell media and placed in the upper portion of both chambers, which were separated by 8 µm membranes (Corning Transwell). The bottom chamber was then treated with 10µg of Phloretin. After six hours, membranes were washed twice in PBS and then fixed in 4 percent formaldehyde. We used crystal violet to colour the cells and then washed them off. The total number of migrating cells (cell number/HPF) was used to calibrate

chemotaxis. Five arbitrary fields per membrane were used to count the number of migrating cells in triplicates[18].

2.7. ELISA-based determination of the inflammatory cytokine TNF-α

Using the protocol that came with the Standard ELISA Kit (Sigma-Aldrich, St. Louis, MO, USA), After adding 100µl of the standard, it was allowed to incubate at room temperature for 2.5 h The sample was aspirated, and it was washed with a 1X wash solution. Next, 100µl of biotinylated detection antibody was added to the mixture. After that, it was again aspirated and washed once more with the 1X wash solution. After adding 100µl of the HRP-Streptavidin solution, the mixture was allowed to incubate at room temperature for half an hour. After that, it was thrown away and washed with a 1X wash solution. Finally,100µlof TMB Reagent was added, and it was incubated at room temperature in the dark for half an hour. After incubation,50µl of stop solutionwas added to each well and the O.D was taken at 450 nm [19].

2.8. Estimation of Connective tissue parameters.

2.8.1. Hydroxyproline

The role of Phloretin in the expression of hydroxyprolinewas measured so that it could be corroborated with collagen synthesis. Firstly, L929 fibroblast cells were grown in culture at the concentration of $1x10^3$ per well and then incubated for 24h at 37°C with 5% CO_2. After incubation the cells were treated with LPS and different concentrations of Phloretin for a period of 24h. After that, the medium that had been cultured was hydrolyzed with 6N HCL at a temperature of 120°C. Then 25µl of the hydrolysate was added into a 96-well plate together with5µl Chloramine-T oxidation buffer. The mixture was then allowed to sit at room temperature for 20min. Then Ehrlich's Reagent was added to each sample and was incubated for 15minsat 65°C. Finally, the absorbance was determined by using a microplate reader at 550nm. The hydroxyproline standard curve was used to determine the concentration of hydroxyproline in the sample. The finding was reported in mg/mL of hydroxyproline[20,21].

2.8.2. Hexosamine

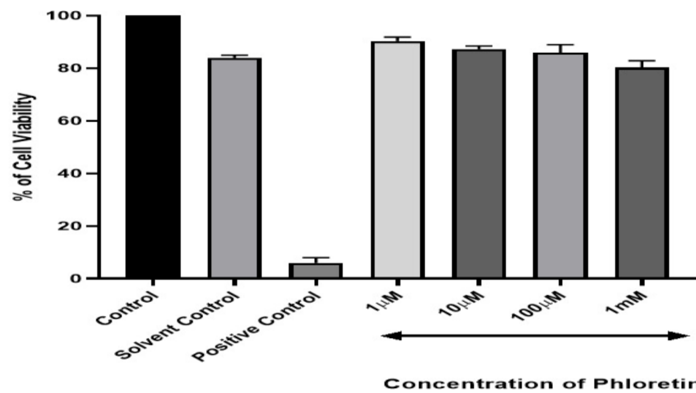
L929 fibroblast cells were grown at a density of $1x10^3$cells per well and incubated for 24h at 37°C with 5% CO_2. After incubation the cells were treated with LPS and different concentrations of Phloretin for a period of 24h. After that, the cultured medium was hydrolyzed with 4N HCL for 8hrs at 98°C, and then neutralized with 4N NaOH to pH 7. It was possible to estimate the granulation tissues hexosamine content with a few minor adjustments[20]. The diluted solution was combined with acetyl acetone solution and heated for 40min at 96°C. After that the mixture was cooled and 96% ethanol was added. Then Ehrlich's Reagent was added to each sample and was incubated for 15minsat 65°C. Finally, the absorbance was determined by using a microplate reader at 530nm[22].

3. Results

3.1. Cell viability of phloretin in L929 Fibroblast

The cytotoxic effect of concentration of phloretin in L929 Fibroblast was evaluated with the help of MTT reduction assay. In this illustration, the viability of the cell is shown while phloretin is

present. **Fig.1** shows that at 1µM to 100µM concentration, phloretin did not affect the viability of the cells, and therefore used for further research.



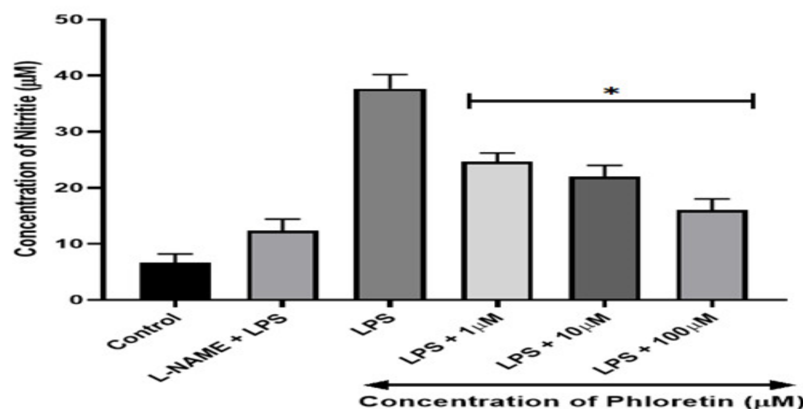
Effect of Phloretin on Cell viability in L929 Fibroblast using MTT assay

L929 Fibroblast were treated with different concentration (1µM, 10µM, 100µM, 1mM) of phloretin for assessing the Cell viability. Untreated cells were treated as control and 0.01% triton x-100 used as positive control. Statistical evaluation was done by one way ANOVA followed by Dunnett's Multiple comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, $P < 0.05$ as compared with control.

3.2. LPS induced anti-inflammatory assay L929 Fibroblast

In this study, Phloretin concentration was evaluated for the inhibition of NO production in the LPS - stimulated L929 fibroblast cells. LPS treatment increased the accumulation of nitrite in the cells. To carry out this experiment, cells were treated with 1µg/ml LPS as well as various concentration of phloretin at the same time.

In untreated cells, we got to see 6.6µM of nitric production where cells that were treated with LPS stimulated the production of nitriteand was 37.6µM but when phloretin was added (i.e., 1µM, 10µM and 100µM), we got to see that at 100µM concentration nitric level significantly reduced to 16µM (**Fig.2**).



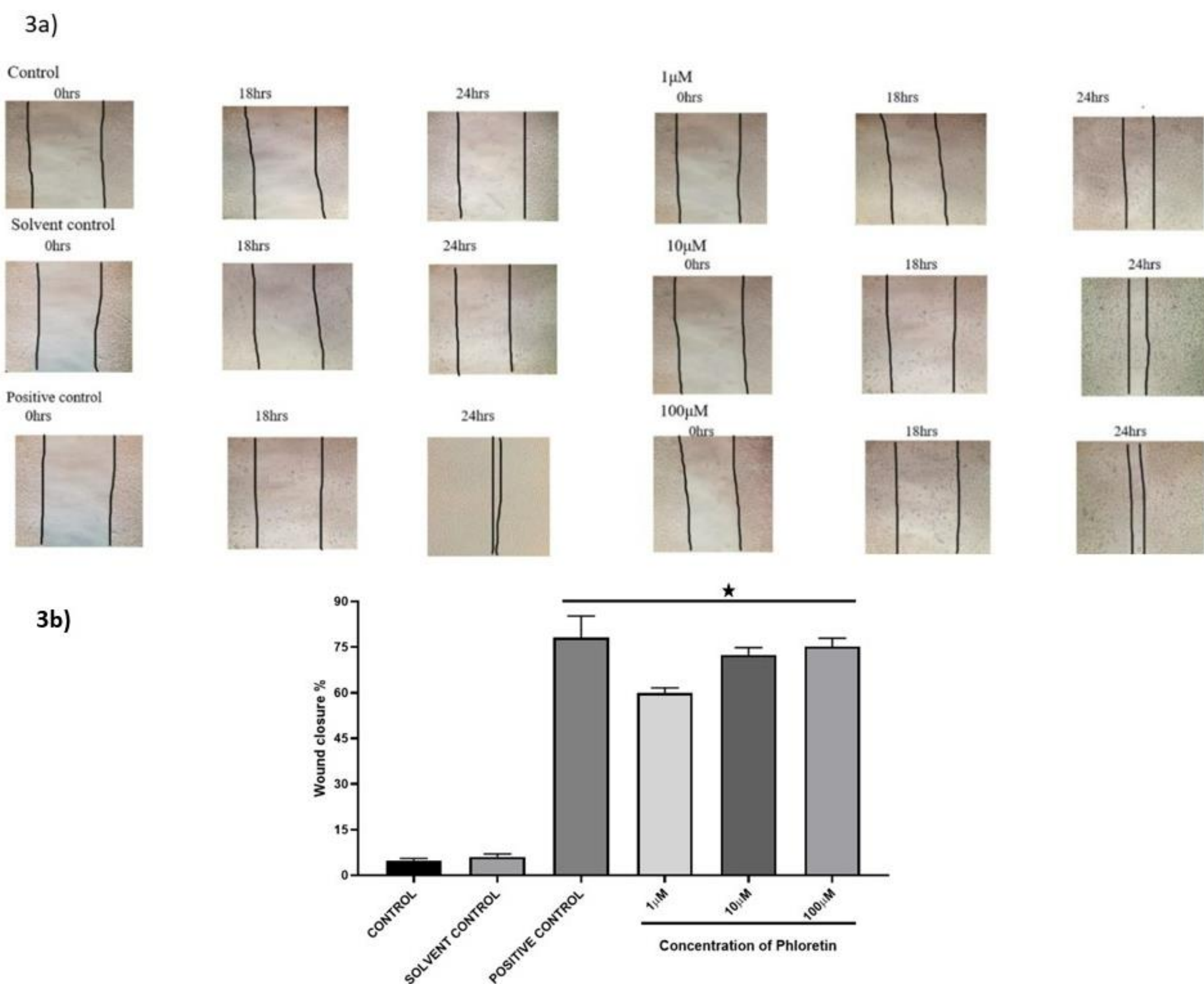
Effects of Phloretin on LPS induced NO production in L929 Fibroblast

LPS induced NO synthesis in L929 Fibroblast was analyzed using Griess method. Statistical evaluation was done by one way ANOVA followed by Dunnett's Multiple comparison test and

expressed as mean ± S.D in triplicates of three independent experiments. *, $P < 0.05$ as compared with LPS.

3.3. Efficacy of Cell Migration of Phloretin in L929 Fibroblast

Scratch assays was used to evaluate the effects of Phloretin on fibroblast migration, which resembles the migration of cells as they heal wounds *in vitro*. Fibroblast migration was measured as a percentage of wound closure in response to phloretin. Mitomycin C which is an antimitotic factor was added to all the well and it acts as inhibitor. Representative images were shown in PDGF-BB was taken as a positive control as it is known for cell proliferation and migration and showed about 79%.While, the phloretin treated groups showed 75% of wound closure(**Fig.3a and 3b).**



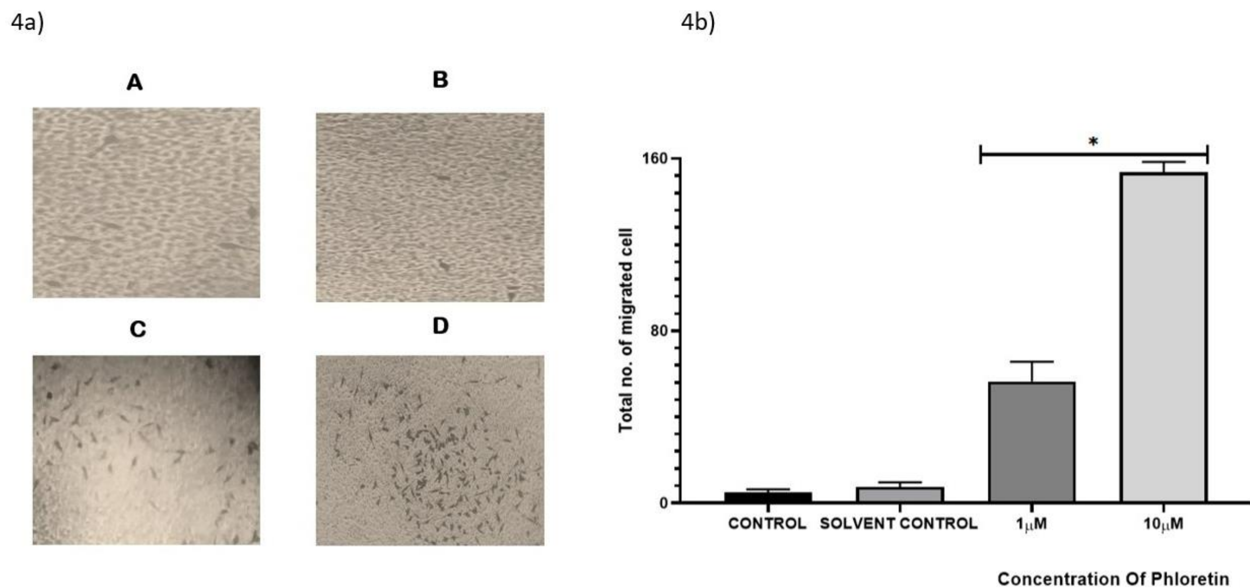
Effect of Phloretin on Cell Migration

Representative scratch assay image to evaluate wound healing in vitro using a confluent monolayer of L929 Fibroblast. Cell migration into the wound was observed in response to an artificial injury. A) single representative area is shown immediately after the wounding and after

18h and 24h. B) Cell migration is represented as % wound closure determined using ImageJ Analyzer from 3 independent experiments. Statistical evaluation was done by one way ANOVA followed by by Dunnett's Multiple comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, $P < 0.05$ as compared with control.

3.4. Chemotaxis analysis by Transwell migration assay

Crystal violet was used to stain the migrated fibroblast on the microporous membrane of Boyden chamber. **Fig.4** showed that Phloretin caused a maximum increase in Chemotaxis. Control cells did not demonstrate any significant increase in migration of fibroblast cell, whereas the maximal responses were obtained at 10Mmconcentration of phloretin only.

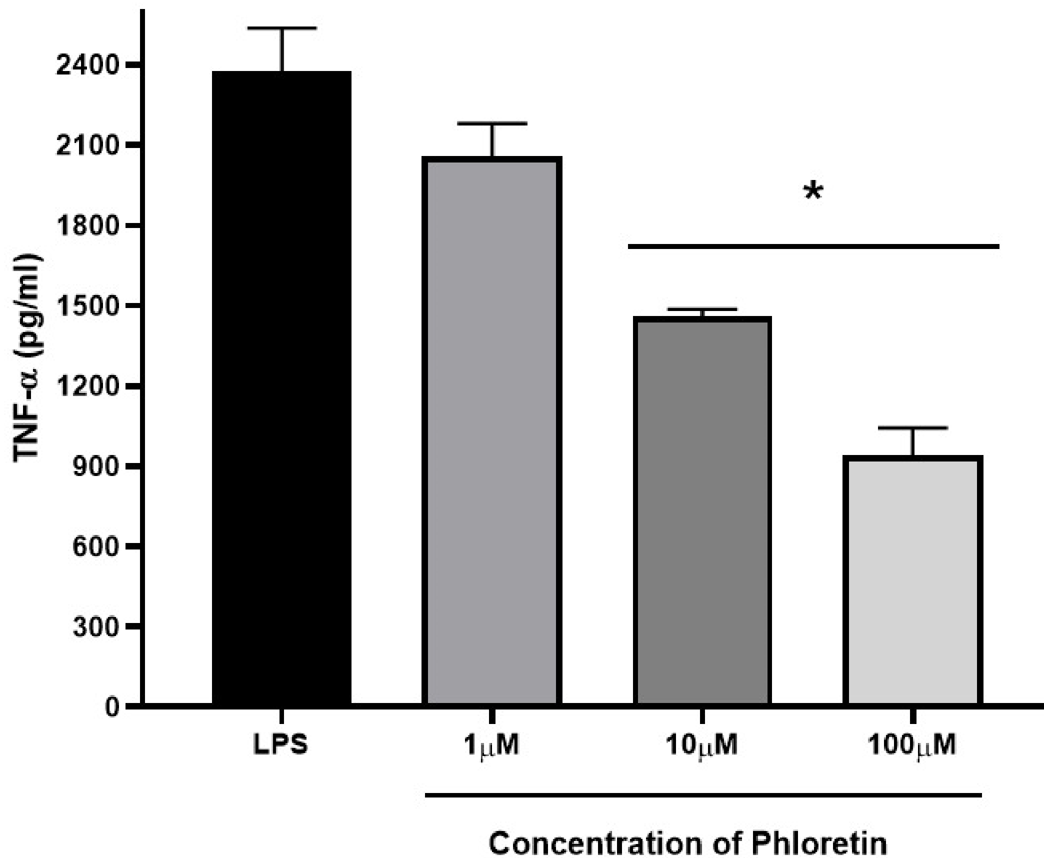


Transwell assay using Modified Boyden chamber

The effect of phloretin on the migration in transwells was evaluated. A) The microphotograph was taken at five different high-power fields (X20) after the cells were stained with crystal violet at a concentration of 0.1 percent. B) Representation of the number of cells that migrated toward the bottom side of the membrane; statistical analysis was performed using one-way ANOVA followed by Dunnett's Multiple comparison test; results were summarized using mean and standard deviation; *p, <0.05 in comparison to the control group.

3.5. Estimation of pro-inflammatory cytokine TNF-α using ELISA.

LPS stimulates the release of pro-inflammatory cytokineslike TNF-α. This cytokine modulates many processes that occur at the wound site, including the activation and growth of keratinocytes, fibroblasts, synthesis of extracellular matrix proteins, and the regulation of immune response.This pro-inflammatory cytokinewas downregulated by Phloretin in dose dependent mannerfrom 1mM to 10mM**Fig.5**



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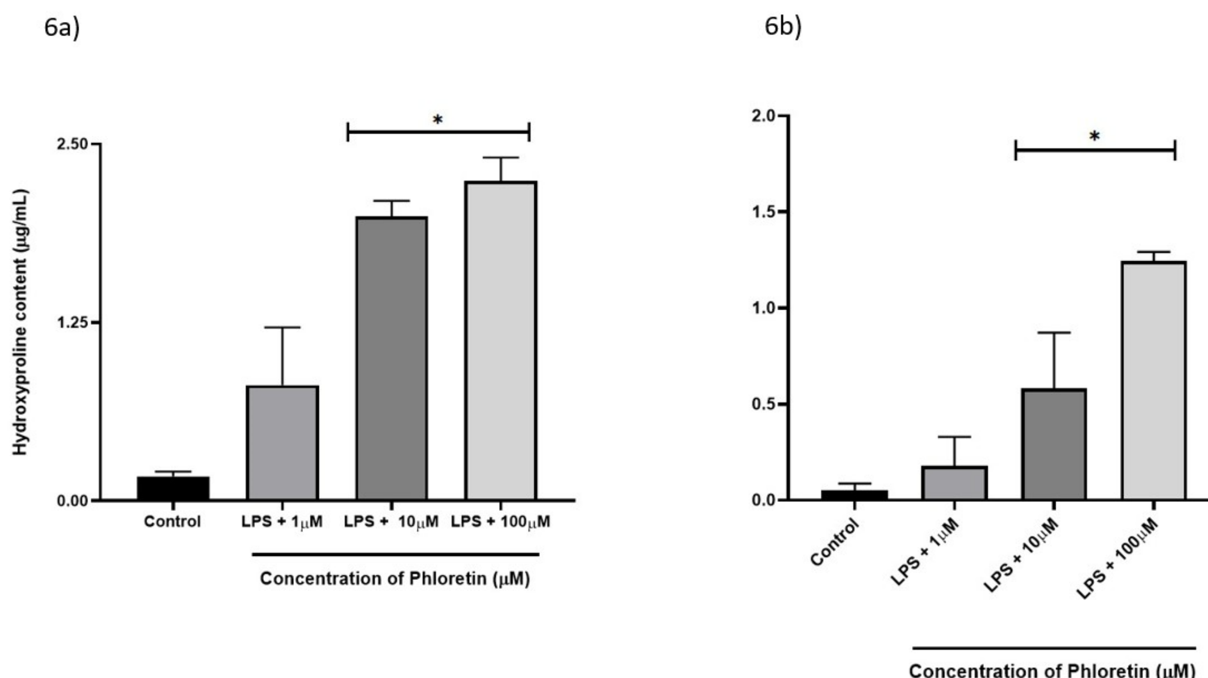
Effect of Phloretin in the production of pro-inflammatory cytokines estimated using ELISA.

Effect of Phloretin in TNF-α expression in LPS treated L929 Fibroblast. TNF-α was measured using enzyme-linked immunosorbent assay (ELISA) on the supernatants of treated cells. L929 Fibroblast were treated with 1µM to 100Mm of Phloretin. Statistical evaluation was done by one way ANOVA followed by Dunnett's Multiple comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, $P < 0.05$ as compared with LPS

3.6. Estimation of Hydroxyproline and Hexosamine Content

The level of hydroxyproline and hexosamine at different concentration of phloretin treated with LPS was measured and was shown in **Fig.6 and Fig. 6b**. It was found that both the Hydroxyproline and Hexosamine content was higher at 10µM and 100µM concentration of phloretin treated with LPS.Increased Hydroxyproline and Hexosamine content was found to have increased phloretin treatment, indicating an increase in cell proliferation and thus an increase in collagen synthesis and rapid healing.



Effects of Phloretin on hydroxyproline and Hexosamine content.

Effect of Phloretin on A) Hydroxyproline and B) Hexosamine content in L929 Fibroblast was measured. Fibroblast that had not been treated were used as control. Statistical evaluation was done by one way ANOVA followed by Dunnett's Multiple comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, $P < 0.05$ as compared with control.

4. Discussion

Both mortality and morbidity can be attributed to wounds, which is a major health issue.Epithelialization, granulation tissue formation, and the remodeling of the extracellular matrix are all involved in wound healing. While the healing process is largely self-perpetuating and delays in developing immunity due to infection. Recent research has shown that phloretin have additional pharmacological functions and biological activities, including antioxidant activity [23,24].

During the tissue formation period of the process of healing, fibroblast proliferation plays a role in the reconstruction of structure and function. A fibroblast's collagen secretion affects the maturation of extracellular matrix into mature dermis [25]. Because fibroblasts are the primary targets in the development of therapeutic drugs, bioactive compounds that have the ability to induce the proliferation of fibroblasts and may be able to speed up the healing process, as was the case in the study that we are reporting here [26].

The MTT assay that was used in this investigation serves multiple purposes, and one of the aims was to identify and determine the optimal concentrations of phloretin so that we could both measure the rate of cell proliferation and influence the metabolic activity of the cells and[27].It was only possible for metabolically active cells to reduce MTT by mitochondrial dehydrogenases in order to determine the viability of the cells. Compared to the control, the Phloretin treated groups

showed no significant difference, proving that phloretin had no toxicity even at the maximum concentration tested.
Fibroblasts are critical in tissue damage and play a role in wound healing by maintaining tissue hemostasis and initiating inflammatory events. They are also involved in the early stages of wound repair. The production of nitric oxide (NO) by these cells is essential to the growth of later developments, including the resolution of inflammation [28]. Cell proliferation and differentiation, modulation of collagen deposition, and wound contraction are all dependent on NO's ability to inhibit cytotoxicity and chemotactic action against different bacteria and tumor cells [29]. Fibroblasts are drawn to a wound to begin the healing process proliferative phase because of a regulation of reactive radical production [30].Inflammatory imbalances can be caused by a powerful stimulus like LPS, which interacts with TLR4, resulting in the production of NO. For this reason, iNOS gene-targeted anti-inflammatory drugs are important in the treatment of inflammatory diseases such as diabetic wound and Alzheimer's disease [11].
Skin cells like fibroblasts move into the injury site and grow rapidly in order to regain skin integrity and produce new granulation tissue during the healing process [31]. Therefore, the appropriate expansion and migration of fibroblast are important considerations in the formation of granulation tissue as the source of new dermis[15]. The scratch assay was utilized in this investigation to determine the effects of phloretin had on fibroblast migration. The scratch assay is a helpful method that mimics the migration of cells during the wound healing process *in vitro*. Here, phloretin with Mitomycin C demonstrated a more rapid recovery compared to the control group.
To be more specific, there was an acceleration in the chemotaxis movement of L929 fibroblast cells following the application of phloretin. This above study demonstrates that phloretin does have the ability to speed up the process of wound healing, control ECM remodeling in an appropriate manner, and promote skin regeneration.
Interleukins (ILs) and TNF-α are two examples of pro-inflammatory cytokines that play an important part in wound repair. These cytokines modulate many processes that occur at the wound site, including the activation and growth of keratinocytes and fibroblasts, the synthesis of extracellular matrix proteins, and the regulation of immune response [29]. While low levels of TNF-α stimulate wound healing by increasing inflammation, it is well-known that higher levels of TNF-α have a detrimental effect on the healing process. TNF-α inhibition could be a successful therapeutic strategy for regulating wound healing, according to this study [32].TNF-α is produced in L929 Fibroblast cells which are pro-inflammatory and play an important part in the cascade of systemic inflammation. To prevent septic shock and inflammation, therapeutical reduction of these cytokines is required. It is necessary to regulate TNF-α acts as an inducer for iNOS. This will allow one to obstruct the sequential response in the cascade of expression of pro-inflammatory mediators, which ultimately results in inflammation. In a dose-dependent manner, phloretin was found to inhibit TNF-α production in L929 fibroblast that had been induced by LPS.

An increase in collagen synthesis occurs in the immediate outcome of an injury, which is why the granulation tissue of a wound is composed primarily of this protein. When collagen is broken down, it releases hydroxyproline and its peptides into the environment. Hydroxyproline is an amino acid that is needed to make collagen, which is the main protein found outside of cells in wound granulation tissue [33]. Hence, collagen turnover can be gauged by measuring hydroxyproline levels. Hemostasis is a major factor in collagen's role in wound healing, as well as in the re-epithelialization of the cellular-matrix and intercellular interactions later in the healing process. Since the wound tissue's increased hydroxyproline content may be the cause of the increased tensile strength and epithelization [34]. The faster the wound heals, the higher the concentration of hydroxyproline in the wound. Hydroxyproline content was found to have increased in LPS treated phloretin, indicating an increase in cell proliferation and thus an increase in collagen synthesis and rapid healing. In the production of new extracellular matrix, matrix molecules such as hexosamine serve as a groundwork. By strengthening electrostatic and ionic interactions with collagen, the glycosaminoglycans stabilize the collagen fibers and may control their final alignment and characteristic size. Protein-protein interactions, which they are capable of binding to and altering, are critical for cellular responsiveness in improvement, homeostasis and disease[35]. Glycosaminoglycans, which hexosamine, were found to be more stable in the presence of LPS treated with Phloretin than in the absence of the supplement. Additionally, collagen contributes structural integrity and homeostasis to the tissue matrix, as well as to the epithelialization process at the end of healing [36]. As a result, the repair tissue and healing pattern are strengthened by increased hydroxyproline and hexosamine synthesis in fibroblast. Ethnotherapeutic claims have been substantiated by the wound contraction and the increased tensile strength and biochemical parameters in healing tissue.

CONCLUSION

The findings of the current study provide empirical evidence in support of the purported benefits of Phloretin in wound healing. In addition to antioxidant, and anti-inflammatory capabilities, the effects of Phloretin on cellular proliferation, migration, and collagen production were able to be determined by a variety of tests. Importantly, the fibroblast cell line was not affected in any way by the cytotoxic effects of the Phloretin. The findings further demonstrated that the production of many pro-inflammatory cytokines can be inhibited by the Phloretin. Consequently, the presence of phloretin performs a regulatory function in the inflammatory mediators when using the LPS-induced paradigm. We also observed that Phloretin increases the Hydroxyproline and hexosamine content which is very crucial for wound healing. This suggests that the phloretin could be used as a possible therapeutic agent for a variety of disorders that are inflammatory in nature. Therefore, it is possible to draw the conclusion that the Phloretin possesses a potential anti-inflammatory action. This activity needs to be further investigated so that it can be used in future clinical therapies.

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Ethical statement
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Competing interests
No potential conflict of interests.
Authors' contribution
All authors have equally contributed with experiments, study consultation, writing, and data handling. Amala Reddy consolidated study materials, experiment design, study validation, supervision, and data analysis.
What is the current Knowledge?
- Phloretin's has good anti-oxidant activity.
- Phloretin can be used in cosmetics

What is new here?
- Envisaging the role of phloretin in LPS induced inflammation.
- Understanding the cell migratory efficacy of Phloretin
- **ABBREVIATIONS**
- MMPs – Matrix metalloproteinase
- ROS – Reactive oxygen species
- ECM – Extracellular matrix
- IL-1: Interleukin-1
- IL-6: Interleukin-6
- TNF-α: Tumor Necrosis Factor-α
- iNOS: inducible nitric oxide synthase
- PDGF: Platelet-Derived Growth Factor
- NF-κB: Nuclear factor kappa light chain enhancer of activated B cells
- LPS: Lipopolysaccharides
- MTT: 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide
- PBS: Phosphate buffered saline
- DMSO: Dimethyl sulfoxide

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