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Research Article
Anti-Inflammatory and Antioxidant Effects of Chrysin Mitigates Diabetic Foot Ulcers

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Abstract

Background and Objective: Diabetes foot ulcer (DFU) is among the worst devastating diabetic complications, frequently leading to amputation and death. Chrysin is a potent flavonoid, with various biological activities including antidiabetic action. However, its efficacy in the prevention of wound healing in DFU is not yet documented. Therefore this study was intended to evaluate the efficacy of chrysin in healing streptozocin-induced diabetic foot ulcers (STZ). **Materials and Methods:** Animals were selected into four groups (n = 10) and the diabetes was induced with STZ (55 mg kg^{-1}). The diabetic animals were made wounds and treated with two different doses (40 and 80 mg kg^{-1} b.wt.) of Chrysin. The boy's weight, food intake, serum glucose, wound contraction rate, HbA1C levels, antioxidants and oxidative stress markers levels were measured. Further, the mRNA expression level of inflammatory markers and protein expression of growth factors were also evaluated. **Results:** Chrysin treatment increased the percentage of wound contraction rate, reduced the HbA1C level and increased the antioxidants and hydroxyproline level and decreased MDA and MOP activity to normal as compared to diabetic wound (DW) rats. Further, Chrysin administration attenuated the elevated mRNA levels of TNF-α, IL-6 and NF-κB mRNA to normal. In addition, the protein level of VEGF and TGF-β1 were markedly improved by Chrysin, implicating its wound healing potential. **Conclusion:** This study demonstrated that the administration of Chrysin could prevent DFU with enhanced wound healing potential.

Key words: Diabetic foot ulcer, wound healing, Chrysin, antioxidants, oxidative stress, inflammation, growth factors

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Globally, diabetes mellitus (DM) imposes a threat to the human population with significant morbidity and mortality. Reports indicate that in 2030, nearly 366 million of the world's population might be affected by DM[1]. In uncontrolled DM individuals may affect with life-threatening multi-organ complications such as retinopathy, nephropathy, cardiomyopathy and diabetic foot ulcers (DFU)[2]. The DFU prevalence ranges from 3-13% globally, with each year's increase rate being 6.4% and the risk increase rate at about 15-25%[3,4]. Nearly 50% of DFU patients are affected with infection leading to higher amputation rates. Reports suggested that DFU is among the main factors causing lower extremity amputations due to non-traumatic conditions among diabetic subjects[5]. In a recent study conducted in China, the average amputation rate is 9.9% among diabetic patients with DFU[3]. The state of hyperglycemia and DFU can provoke oxidative stress and elevates the free radical generation and minify the antioxidant defence[6]. Further, chronic exposure to hyperglycemia environment to cells and tissues leads to the destruction of proteins and lipids and enhances the risk of wound ulcers and further delay in wound healing[7].

Disruptions in wound healing, such as elevated inflammatory cytokines levels and impairments in the generation of growth regulators and cell signalling molecules, are strongly linked to delayed wound healing during diabetes[8]. Further, elevated oxidative stress, delay in epithelization, collagen production, angiogenesis and alerted endothelial cell functions are the cardinal factors involved in wound pathophysiology[9]. Previously, several studies documented that the reduction of the Transforming Growth Factor (TGF) and Vascular Endothelial Growth Factor (VEGF) may interrupt curing activity in diabetic rats[10]. According to previous preclinical studies, low concentrations of the Transforming Growth Factor (TGF) and Vascular Endothelial Growth Factor (VEGF) have a significant role in decreasing wound healing in diabetic rats.

The Nuclear Factor κB (NF-κB) is one of the important molecules that orchestrate and contribute significantly to the progression of end-organ destruction in DM. Prior studies have demonstrated that inhibiting the NF-B pro-inflammatory signalling pathway slows wound healing[11]. Furthermore, oxidative stress apoptosis contributes significantly to delayed wound healing in diabetes patients[12]. Albeit, mounting advancements in pharmaceutical research have been proposed, the therapeutic strategy for the management of DFU has not been improved to date. Currently, DFU has approved the cell and growth factor techniques. However, it elicits adverse effects with increased economic burden and thus it serves as a barrier to the treatment[10]. Plant-derived chemicals are gaining popularity and wide attention in the therapy of diabetes and its related consequences. A recent systematic analysis shows that medicinal plants with antioxidant potential serve as a promising adjuvant therapy to accelerate wound healing in DFU patients[13]. A naturally occurring flavonoid called Chrysin (5,7-dihydroxyflavone) may originate in propolis, honey and plant exudates. Chrysin exerts effective antioxidant and hypolipidemic activity and is regarded as a potential antidiabetic agent with cardiac and hepato-protective properties[14].

Therefore, this study was intended to evaluate the efficacy of Chrysin in healing Streptozocin-induced diabetic rats via its mechanisms.

MATERIALS AND METHODS

Study area: The present study was carried out in the South Area of Guang'anmen Hospital from January to April, 2022.

Chemicals: Streptozocin (STZ) and Chrysin were obtained from Sigma-Aldrich Corp (USA). The other analytical-grade chemicals were obtained from Merck (USA).

Animals: For this investigation, 40 adult male Wistar rats (160-180 g) were procured from the Institution's Animal House facility. The test animals were kept in isolated, wide, clean cages with a constant temperature of 23±1°C and exposed to a 12 hrs dark-light sequence during the testing period. The animals were allowed 7 days to adapt to laboratory circumstances. The experiments were performed by the Institute's Ethics Committee and the Regulations for the Protection and Handling of Laboratory Animals (USA) (Reg. No. 36445/2021/CPC/FTULC/12.09.2021).

Diabetes induction and wound excision model: The animals developed diabetes after receiving a single intraperitoneal administration of STZ (55 mg kg^{-1}) suspended in 0.1 M citrate buffer (pH 4.4). During the same time, the control group was given the same volume of vehicle (citrate buffer with distilled water). After blood was drawn through retro-orbital injection, the serum glucose concentrations were measured using the GOD-POD (glucose oxidase-peroxidase) approach. The DFU was induced in rats using an excision wound approach, as described in a prior publication[15]. The selected diabetic animals were anaesthetized intraperitoneally with ketamine (80 mg kg^{-1}) and xylazine (12 mg kg^{-1}). On the dorsal side of the right paw, a rectangular region of skin removal with conventional dimensions of 2×5 mm was produced.

Experimental design: After acclimation, 40 animals were randomly assigned into 4 groups (n = 10), as follows:

- **NDWC group:** Non-diabetic wounded control rats (NDWC) received vehicle (citrate buffer in distilled water)
- **DW group:** Diabetic wounded rats (DW) received vehicle (citrate buffer in distilled water)
- **DW+Chrysin (40 mg kg^{-1}) group:** Diabetic wounded rats (DW) received Chrysin at the dose of 40 mg kg^{-1} through the oral route
- **DW+Chrysin (80 mg kg^{-1}) group:** Diabetic wounded rats (DW) received Chrysin at the dose of 80 mg kg^{-1} through the oral route

The Chrysin doses were selected based on the previous literature published by Usai *et al.*[14].

The animals' food consumption and body weight were noted. At the end of the trial, after 16 days, the animals were decapitated by cervical decapitation after being given phenobarbital sodium (35 mg kg^{-1}, i.p.) as an anaesthetic. Heparinised tubes were used to collect the blood from the jugular vein and the serum was centrifuged and collected for the determination of biochemical markers level. The wound tissues were collected, weighed (100 mg) and homogenised in a pre-chilled Tris-HCl buffer with 10% w/v before being kept at (-80°C) for the analysis of several biochemical markers.

Measurement of wound healing: On days 1, 8 and 16, the wound regions were carefully measured. The formulas listed below were used to calculate the percentage of wound closure (WC (%))[16]:

$$\text{Wound closure (\%)} = \frac{\text{Initial wound area-Would area on Nth day}}{\text{Initial wound area}} \times 100$$

The graph was prepared by plotting WC (%) vs duration (days) using SPSS analytical software and the time required to close the wound diameter by 50% (CT_{50}) was calculated.

Estimation of glucose, insulin and glycated haemoglobin (HbA1C) levels: Glucose, insulin and glycated haemoglobin levels were measured using commercial kits following the manufacturer's guidelines.

Estimation of biochemical markers: The biochemical analysis, such as reduced GSH, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in skin tissue, were tested using a commercially available kit from Sigma Aldrich, USA. Further, the skin tissue homogenate levels of lipid peroxidation markers, malondialdehyde (MDA), myeloperoxidase (MPO) and hydroxyproline levels were estimated using the kits from Sigma Aldrich, USA.

Estimation of inflammatory markers: The levels of pro-inflammatory cytokines, Tumour Necrosis Factor (TNF- α) and Interleukin-6 (IL-6) in blood serum were determined using an ELISA kit and the manufacturer's instructions.

Western blot analysis: TGF-β1 and VEGF levels in cardiac tissue were determined using Western blot analysis. Total proteins from cardiac tissues were extracted with RIPA lysis buffer (Bio-Tek, USA). Protein concentrations were determined by SDS-PAGE, with TGF-β1 and VEGF getting 10% of the SDS-PAGE concentration. As primary antibodies, rabbit anti-TGF- β1 (1:800) and rabbit anti-VEGF (1:800) were utilized. The secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit, was incubated at 37°C for 2 hrs. Protein bands were observed through ECL kits and analysed using the FR-200 system (Shanghai FURI Technology).

mRNA expression of TNF-α, IL-6 and NF-κB by RT-PCR: The skin tissue sample of total RNA was purified using the RNA out method as per the instruction provided by Takara. The cDNAs were prepared using 1 µg of total RNA from the prepared sample.

Following primers were used:

- **IL-6 (509 bp):** 5′ TCCTACCCCAACTTCCAATGCTC 3′ (forward), 5 TTGGATGGTCTTGGTCCTTAGCC 3 (reverse)
- **TNF-α:** 5′-CACCATGAGCACAGAAAGCA-3 forward, 5′ TAGACAGAAGAGCGTGGTGG-3 reverse
- **NF-κB (P65) (381 bp):** 5′ CCTATCCACGACAACCTTGC 3′ (forward), 5′ CATAGATGCTGCTGACCCAAC 3 (reverse)
- **β-actin (493 bp):** 5′ -GTGGGGCGCCCAGGCACCA-3′ (forward) and 5′-GCTCGGCCGTGGTGGTGAAGC-3′ (reverse)

The reactions were performed in 30 cycles at 95°C for 30 sec of denaturing, 57°C for 30 sec of annealing and 72°C for 1 min of extension. The Fluor Chen 2.0 computer-based image analysis system was used to calculate the integrated densities value (IDV).

Statistical analysis: The statistical tool SPSS v. 24 was used for the analysis, one-way ANOVA and Dunnett's test were analyzed. The outcomes values were expressed as Mean±Standard Error Mean (SEM). The 'n' represents the count of animals in each group. The statistical variation was set as a $p<0.05$.

RESULTS

Impact of Chrysin on body weight and food consumption: In this study, DW group animals expressed reduced weight considerably faster ($p<0.05$) than the other tested groups (Fig. 1). The DFU animals were treated with Chrysin at doses of 40 and 80 mg kg^{-1} b.wt., increased their body weight significantly more than DFU group animals ($p<0.05$). Meanwhile, animals in the DW group consumed considerably more food than other tested groups ($p<0.05$) (Fig. 2). At the dosages of 40 and 80 mg kg^{-1} of chrysin, food consumption was considerably reduced in DFU animals when compared to the DW group ($p<0.05$).

Effect of Chrysin on the levels of glucose, insulin and HbA1C: In the study, glucose and HbA1C levels were considerably increased ($p<0.05$) in the DW group, but the insulin level was reduced in the NDWC group. The experimental results were summarized in Table 1. At the dosages of 40 and 80 mg kg^{-1} of chrysin, the glucose and HbA1C were considerably reduced and elevated insulin levels were to normal.

Wound healing effect of Chrysin in diabetic animals induced by STZ: In comparison to the test groups, the DW group's percentage of wound closure was significantly lower (-21.65 ± 2.76 vs 30.12 ± 3.54, $p<0.05$). The results were shown in Table 2. Treatment of diabetic wounded rats with Chrysin at the dose of 40 mg kg^{-1} (76.87 ± 6.76 vs -20.65 ± 2.76, $p<0.05$) and 80 mg kg^{-1} (92.14 ± 8.34 vs -20.65 ± 2.76, $p<0.05$) displayed higher wound contraction rate as compared to DW group and it was found to be significant. The CT_{50} is the time required for the 50% closure of the wound. The CT_{50} in Chrysin-treated animals was considerably lower than in the DW group, thus enhancing wound healing.

Chrysin improves the antioxidant defense and inhibits the oxidative in diabetic wounded rats: The diabetic wound tissue levels of GSH, SOD, CAT and GPx in the DW group were considerably lower than in other tested groups. Furthermore, MPO and MDA activity rose considerably in the DW group. Further, the activity of hydroxyproline was lowered in the DW group compared to other tested groups. Meanwhile, treatment with Chrysin at dosages of 40 and 80 mg kg^{-1} considerably improved antioxidant and hydroxyproline levels while lowering MDA and MPO levels ($p<0.05$) (Table 3).

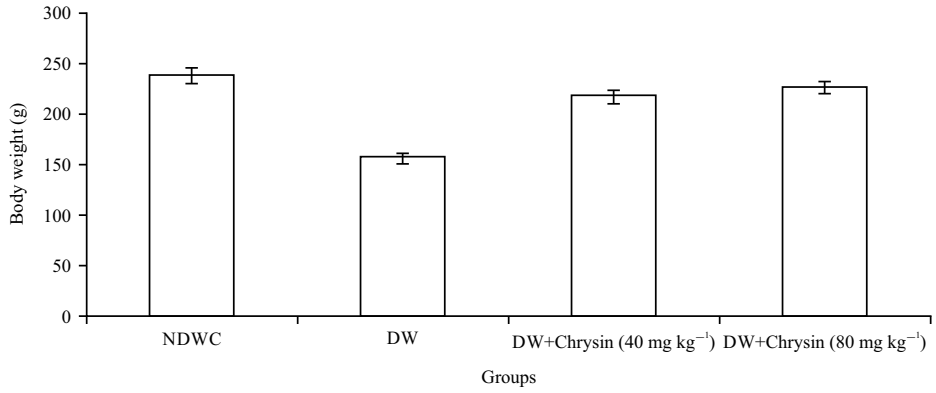

Fig. 1: Effect of Chrysin on body weight

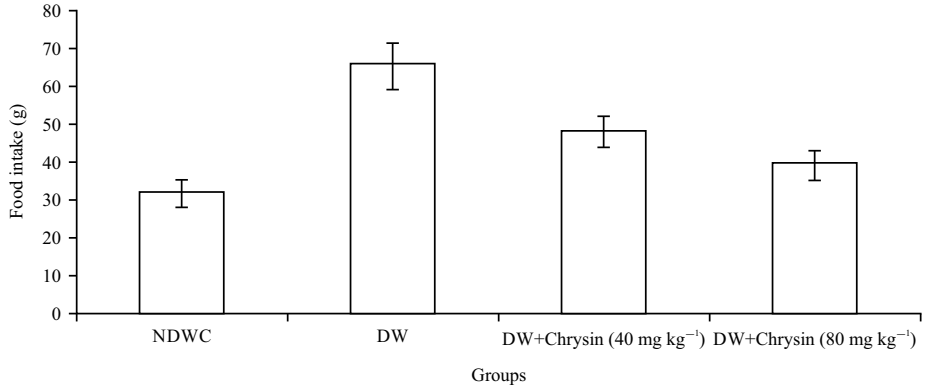

Fig. 2: Effect of Chrysin on food intake

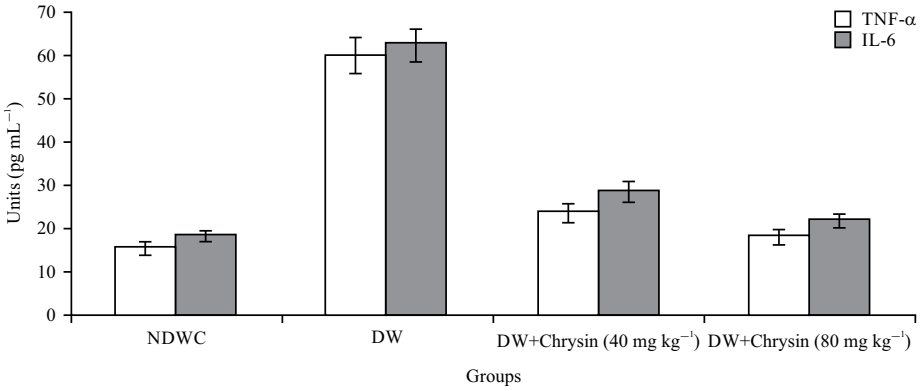

Fig. 3: Levels of the proinflammatory cytokine

Table 1: Effect of Chrysin treatment on the level's glucose, HbA1C and insulin in diabetic wounded rats

Groups	Glucose (mg dL^{-1})	HbA1C (%)	Insulin (mg L^{-1})
NDWC	96.12±8.43	4.24±0.12	2.65±0.16
DW	390.76±16.32[a]*	11.54±0.92[a]*	0.82±0.05[a]*
DW+Chrysin (40 mg kg^{-1})	170.45±11.65[b]*	6.84±0.23[b]*	1.96±0.09[b]*
DW+Chrysin (80 mg kg^{-1})	152.65±12.87[b]*	5.43±0.42[b]*	2.34±0.12[b]*

Data were represented as Mean±SD (n = 10), [a]Comparison between DW vs NDWC, [b]Comparison between DW+Chrysin (40 and 80 mg kg^{-1}) vs DW, *p<0.05 (significant), DW: Diabetic wounded and NDWC: Non-diabetic wounded control

Table 2: Wound healing activity of Chrysin in STZ diabetic rats

Groups	Wound contraction (%)	CT_{50} (Days)
NDWC	30.12±3.54	10.76±1.65
DW	-21.65±2.76[a]*	40.12±6.24[a]*
DW+Chrysin (40 mg kg^{-1})	76.87±6.76[b]*	20.87±4.12[b]*
DW+Chrysin (80 mg kg^{-1})	92.14±8.34[b]*	18.32±5.98[b]*

Data were represented as Mean±SD (n = 10), [a]Comparison between DW vs NDWC, [b]Comparison between DW+Chrysin (40 and 80 mg kg^{-1}) vs DW, *p<0.05 (significant), DW: Diabetic wounded and NDWC: Non-diabetic wounded control

Table 3: Effect of Chrysin on antioxidant and oxidative stress markers in DFU rats

Groups	GSH	SOD	CAT	MDA	MPO	HDP
NDWC	7.15±0.97	4.65±0.12	2.78±0.08	5.24±0.87	0.76±0.04	3.87±0.43
DW	2.45±0.08[a]*	1.76±0.06[a]*	0.96±0.01[a]*	21.54±4.65[a]*	2.36±0.08[a]*	0.96±0.06[a]*
DW+Chrysin (40 mg kg^{-1})	5.28±0.78[b]*	3.43±0.09[b]*	1.85±0.06[b]*	8.42±1.76[b]*	1.28±0.06[b]*	2.32±0.24[b]*
DW+Chrysin (80 mg kg^{-1})	6.78±0.86[b]*	4.12±0.15[b]*	2.52±0.09[b]*	6.12±0.96[b]*	0.95±0.08[b]*	3.18±0.76[b]*

Data were represented as Mean±SD (n = 10), [a]Comparison between DW vs NDWC, [b]Comparison between DW+Chrysin (40 and 80 mg kg^{-1}) vs DW, *p<0.05 (significant), DW: Diabetic wounded, NDWC: Non diabetic wounded control Units, SOD: U mg^{-1} protein, GSH: µg mg^{-1} protein, CAT: U mg^{-1} protein, MDA: nmole mg^{-1} protein, MPO: U mg^{-1} protein and HDP (Hydroxyproline): µg mg^{-1} tissue

Chrysin effectively inhibits the levels of the proinflammatory cytokine in wound skin tissue: The TNF-α and IL-6 serum concentrations in the DW group were significantly higher (p<0.05) than in the other tested groups (Fig. 3). At the dosages of 40 and 80 mg kg^{-1} of chrysin, levels of inflammatory markers were significantly decreased in the DW group.

Effect of Chrysin on TGF-β1 and VEGF western blot protein expression in streptozocin-induced DFU rats: In this study, TGF-β1 and VEGF protein expressions were comparatively reduced in DW and NDWC groups (Fig. 4a). In comparison to the DW group, the TGF-1 and VEGF levels were considerably greater (p<0.05) at the doses of 40 and 80 mg kg^{-1} of chrysin (Fig. 4b).

Effect of Chrysin on TNF-α, IL-6 and NF-κB mRNA expression in streptozocin-induced DFU rats: In this study, RT-PCR was done to evaluate the levels of inflammatory markers in DFU rats. When compared to the DW group, the cytokine-treated groups showed promising results in terms of TNF- α, IL-6 and NF-κB downregulation (Fig. 5a). Compare to another tested group, TNF- α, IL-6 and NF-κB expression was considerably high (p<0.05) in the DW group (Fig. 5b).

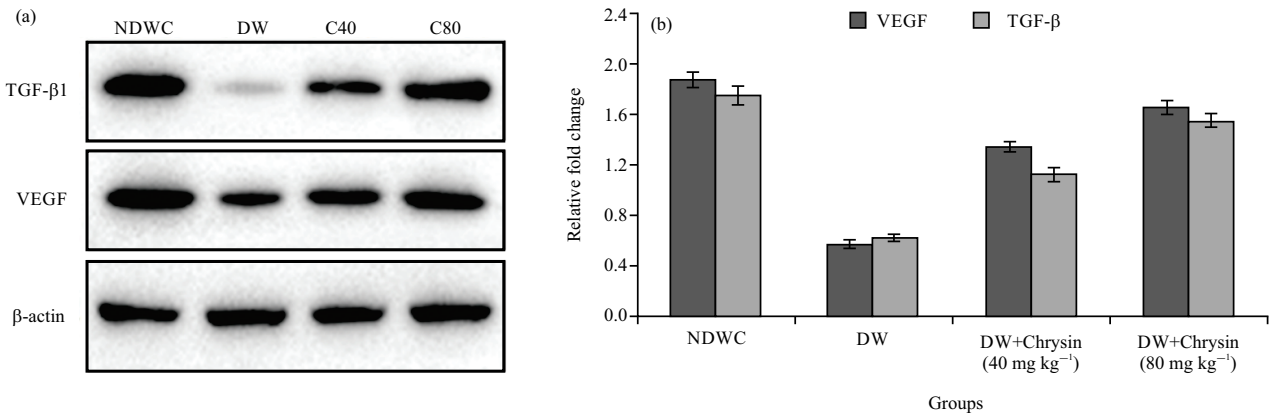

Fig. 4(a-b): Effect of Chrysin on TGF-β1 and VEGF western blot protein expression in DFU rats

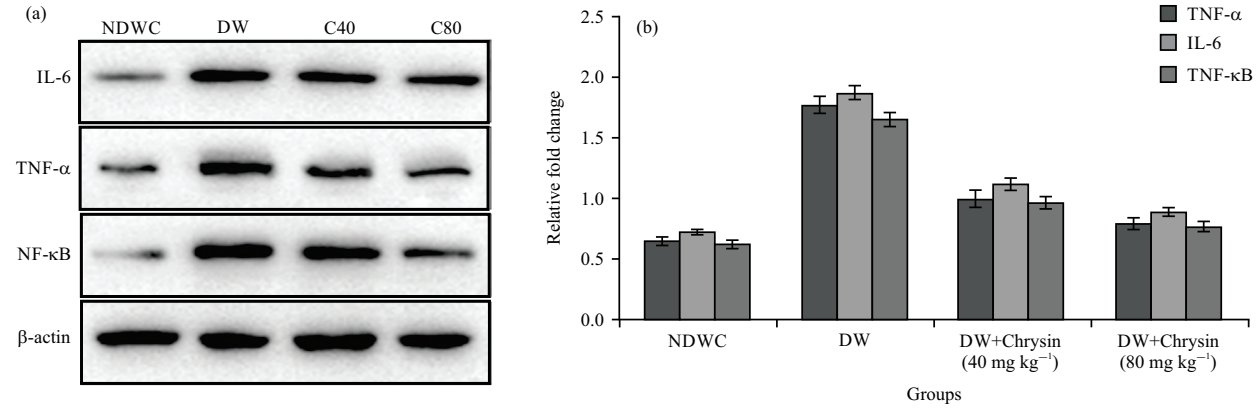

Fig. 5(a-b): Effect of Chrysin on TNF-α, IL-6 and NF-κB mRNA expression in DFU rats

DISCUSSION

The DFU is one of the major complications among DM patients and the aetiology of this disease involves complex pathways. Elevated blood glucose levels reduce endothelial cell functions, weaken the immune system, elevate the chance of microbial infections and decrease the synthesis of growth factors such as TGF and VEGF, respectively[17].

Mounting factors are responsible for delayed wound healing in diabetes patients such as impairment in the blood flow, insulin, altered cell membrane permeability, reduced collagen synthesis, increased oxidative stress, inflammation and apoptosis[18]. The DFU care options now include human growth factor treatment, oxygen treatments and bioengineered tissue. But in a real clinical scenario, treatment of DFU is difficult and it leads to lower limb amputation in the majority of the patients[19]. Herein, in this research, the efficacy of Chrysin in the prevention of DFU in streptozocin-induced diabetic rats was reported.

Previous studies show excessive body weight loss and increased hunger episodes were the early clinical manifestation of diabetes. In this study, the DFU rats displayed decreased body weight and increased food intake and treatment with Chrysin effectively improved the body weight and food intake[14].

Elevated glucose levels impair wound healing by reducing neutrophil-assisted chemotaxis and thus enhance the infectious process. The clinical events encountered in DFU were mainly due to the elevated levels of glycated haemoglobin and this reflects the uncontrolled glycemic status over a certain period. Reports showed that advanced glycation end products can cross-link with fibronectin and thus delay wound healing in diabetes[20]. In this study, DFU rats displayed marked elevation of HbA1C and treatment with Chrysin significantly reduced the HbA1C level to normal. Previous research has revealed that HbA1c levels were substantially connected to wound healing rate, with wounds with higher levels of HbA1C healing slower[21]. The results of this experiment clearly showed that chrysin can improve wound healing by lowering HbA1c levels.

Fibroblasts orchestrate a vital role in faster wound healing by transforming the granular tissue into myofibroblasts which further enhances collagen production. In this study, DFU rats displayed a reduced percentage of wound contraction rate and increased days to reach CT_{50}. Chrysin-treated DFU rats displayed a faster wound contraction rate and decreased time to reach CT_{50}. The previous study showed that Chrysin treatment markedly enhanced collagen synthesis in human dermal fibroblasts[22].

Preclinical studies revealed that oxidative stress is the major contributor to the aetiology of diabetic wounds as a result of free radicals derived from neutrophils-derived oxidants and MPO activity and in combination, they enhance tissue damage in the wound area[23]. Thus enhanced production of free radicals induces cytotoxicity and delays wound healing. So, the prevention or reducing free radical generation is the cardinal mechanism in chronic wound healing. MPO and MDA (lipid peroxidation indicators) were found to be increased in diabetic rat wound skin tissue. In contrast, antioxidant levels such as GSH, SOD and CAT were decreased in diabetic rat wound skin tissue, which might be owing to the use of antioxidants to combat free radicals created during chronic wounds[24]. However, Chrysin treatment decreased MDA and MPO levels while increasing antioxidant levels to normal, restoring the antioxidant defense system in the wound region. Chrysin's free radical scavenging action is mostly owing to the double bond nature of C2-C3 and the presence of the carbonyl group at the C4 position[25]. In addition, the presence of hydroxyl group at C5 and C7 was also attributed to its antioxidant activity.

In DFU, the expression of chemokines, cytokines and their receptors is important at different stages of wound healing. TNF-α increases apoptosis and lowers angiogenesis, tissue proliferation, differentiation and migration in chronic wounds.

Previous reports showed that the wound healing rate is decreased due to increased levels of proinflammatory cytokines such as TNF-α and IL-6 due to the triggering of apoptosis and decreased movement of fibroblast[26]. Further, TNF-α also triggers the release of matrix metalloprotease (MMP) which induces the matrix protein and growth factors degradation and thus delay the wound healing process[27]. In hyperglycemic circumstances, NF-κB activation causes a flood of inflammatory cytokines to be released. NF-κB acts as a transcription inducer of oxidative stress and enhances the inflammatory process and delays wound healing[28].

In the current study, DFU animals had higher levels of TNF-α and IL-6 in their serum, as well as higher levels of TNF-α, IL-6 and NF-κB mRNA expression in wound tissue homogenate. Treatment with Chrysin effectively restored the increased level of TNF-α and IL-6 to normal and also showed a downregulation of mRNA expression of inflammatory mediators which is in line with the earlier report[29].

Angiogenesis is a multistage process that involves the transformation of blood vessels into endothelial cells. The development of new blood vessels is not only oxygen dependent but also needs various signalling pathways which give instructions to cells and tissues. Mounting molecular pathways are involved in delayed wound healing during diabetes. Biological mechanisms such as increased proinflammatory cytokine activity, reduced angiogenic activity, decreased keratinocyte and fibroblast migration and proliferation and altered synthesis of healing-related growth factors all have an impact on wound healing[30]. The vital molecules involved in wound healing are VEGF and collagen. Previous reports show that the activity of VEGF is decreased at the diabetic wound site and leads to a delay in wound healing[19]. Another key molecule involved in wound healing is TGF-β, which is produced throughout the early stages of healing. The TGF-β controls tissue remodelling during the final phase of wound healing by replacing collagen type III with collagen type I and extracellular matrix formation and granular tissue regeneration[31]. Previous reports showed that impaired or downregulation in TGF-β leads to delayed wound healing during diabetes[19]. Likewise, in the present study, DFU rats showed downregulation in the protein expression of VEGF and TGF-β1 and treatment. Chrysin treatment showed significant upregulation in protein expression of VEGF and TGF-β1 and earlier reports showed that topical application of propolis which contains Chrysin as one of the main components effectively increased the TGF-β1 signalling[32].

Reduced collagen deposition is one of the primary mechanisms for delayed wounds and it is evident when there is a low level of hydroxyproline in wound tissue. Chrysin treatment effectively increased the hydroxyproline levels and ensured wound healing, which is in line with an earlier report[33]. The current findings show that administration of Chrysin significantly lowered blood glucose levels, HbA1C and oxidative stress while enhancing antioxidant status. Chrysin also aided wound healing by decreasing proinflammatory cytokine levels while raising VEGF, TGF-1 and hydroxyproline protein levels. Thus, chrysin has the potential to be used as a therapy for diabetic foot ulcers and chronic wounds. However, more investigation is necessary to completely understand the gene expression and molecular mechanisms involved in chronic wound healing.

CONCLUSION

Based on the study outcome, it was concluded that Chrysin treatment significantly decreased the blood glucose levels, HbA1C and oxidative stress and improved the antioxidant status. Chrysin also improved wound healing by decreasing the levels of the proinflammatory cytokine and boosting the protein levels of VEGF, TGF-β1 and hydroxyproline. Thus, Chrysin can be used as a potential agent in the management of DFU and chronic wound in diabetic patients.

SIGNIFICANCE STATEMENT

Diabetes foot ulcer (DFU) is one of the most serious diabetes complications, often resulting in amputation and death. Chrysin is a powerful flavonoid with a variety of biological properties, including anti-diabetic activity. However, its effectiveness in preventing wound healing in DFU has yet to be shown. Therefore, this study was carried out to determine the effectiveness of the Chrysin used to heal streptozocin-induced diabetic foot ulcers (STZ). According to the findings of the study, chrysin administration dramatically reduced blood glucose levels, HbA1C and oxidative stress while improving antioxidant status. Chrysin also promoted wound healing by lowering proinflammatory cytokine levels and increasing VEGF, TGF-β1 and hydroxyproline protein levels. Thus, chrysin has the potential to be employed as a treatment for DFU and chronic wounds in diabetic patients.

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REFERENCES

1. Roglic, G., 2016. WHO global report on diabetes: A summary. Int. J. Noncommunicable Dis., 1: 3-8.
2. Cade, W.T., 2008. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys. Ther., 88: 1322-1335.
3. Yazdanpanah, L., H. Shahbazian, I. Nazari, H.R. Arti and F. Ahmadi *et al*., 2018. Incidence and risk factors of diabetic foot ulcer: A population-based diabetic foot cohort (ADFC study)-two-year follow-up study. Int. J. Endocrinol., Vol. 2018. 10.1155/2018/7631659.
4. Lu, Q., J. Wang, X. Wei, G. Wang, Y. Xu, Z. Lu and P. Liu, 2020. Cost of diabetic foot ulcer management in China: A 7-year single-center retrospective review. Diabetes Metab. Syndr. Obesity: Targets Ther., 13: 4249-4260.
5. Zhang, P., J. Lu, Y. Jing, S. Tang, D. Zhu and Y. Bi, 2017. Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. Ann. Med., 49: 106-116.
6. Ugwu, E., O. Adeleye, I. Gezawa, I. Okpe, M. Enamino and I. Ezeani, 2019. Predictors of lower extremity amputation in patients with diabetic foot ulcer: Findings from MEDFUN, a multi-center observational study. J. Foot Ankle Res., Vol. 12. 10.1186/s13047-019-0345-y.
7. Bajaj, S. and A. Khan, 2012. Antioxidants and diabetes. Indian J. Endocrinol. Metab., 16: S267-S271.
8. Giri, B., S. Dey, T. Das, M. Sarkar, J. Banerjee and S.K. Dash, 2018. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. Biomed. Pharmacother., 107: 306-328.
9. Dunnill, C., T. Patton, J. Brennan, J. Barrett and M. Dryden *et al*., 2017. Reactive oxygen species (ROS) and wound healing: The functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Int. Wound J., 14: 89-96.
10. Kim, Y.W. and T.V. Byzova, 2014. Oxidative stress in angiogenesis and vascular disease. Blood, 123: 625-631.
11. Miricescu, D., S.C. Badoiu, I.I. Stanescu-Spinu, A.R. Totan, C. Stefani and M. Greabu, 2021. Growth factors, reactive oxygen species, and metformin-promoters of the wound healing process in burns? Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22179512.
12. Liu, T., L. Zhang, D. Joo and S.C. Sun, 2017. NF-κB signaling in inflammation. Signal Transduction Targeted Ther., Vol. 2. 10.1038/sigtrans.2017.23.
13. Kunkemoeller, B. and T.R. Kyriakides, 2017. Redox signaling in diabetic wound healing regulates extracellular matrix deposition. Antioxid. Redox Signaling, 27: 823-838.
14. Usai, R., S. Majoni and F. Rwere, 2022. Natural products for the treatment and management of diabetes mellitus in Zimbabwe-A review. Front. Pharmacol., Vol. 13. 10.3389/fphar.2022.980819.
15. Samarghandian, S., T. Farkhondeh and F. Samini, 2017. Honey and health: A review of recent clinical research. Pharmacogn. Res., 9: 121-127.
16. Chen, L., R. Mirza, Y. Kwon, L.A. DiPietro and T.J. Koh, 2015. The murine excisional wound model: Contraction revisited. Wound Repair Regener., 23: 874-877.
17. Pillai, S.I., P. Palsamy, S. Subramanian and M. Kandaswamy, 2010. Wound healing properties of Indian propolis studied on excision wound-induced rats. Pharm. Biol., 48: 1198-1206.

18. Florindo, H.F., R. Kleiner, D. Vaskovich-Koubi, R.C. Acúrcio and B. Carreira *et al*., 2020. Immune-mediated approaches against COVID-19. Nat. Nanotechnol., 15: 630-645.
19. Patel, S., S. Srivastava, M.R. Singh and D. Singh, 2019. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomed. Pharmacother., Vol. 112. 10.1016/j.biopha.2019.108615.
20. Pouget, C., C. Dunyach-Remy, A. Pantel, A. Boutet-Dubois and S. Schuldiner *et al*., 2021. Alternative approaches for the management of diabetic foot ulcers. Front. Microbiol., Vol. 12. 10.3389/fmicb.2021.747618.
21. Casadei, G., M. Filippini and L. Brognara, 2021. Glycated hemoglobin (HbA1c) as a biomarker for diabetic foot peripheral neuropathy. Diseases, Vol. 9. 10.3390/diseases9010016.
22. Christman, A.L., E. Selvin, D.J. Margolis, G.S. Lazarus and L.A. Garza, 2011. Hemoglobin A1c predicts healing rate in diabetic wounds. J. Invest. Dermatol., 131: 2121-2127.
23. Hassan, R., D. Sabry and A.A. Rabea, 2022. Assessment of ultra-structure, viability and function of lipopolysaccharides-stimulated human dermal fibroblasts treated with chrysin and exosomes (*in vitro* study). Saudi Dent. J., 34: 346-354.
24. Deng, L., C. Du, P. Song, T. Chen, S. Rui, D.G. Armstrong and W. Deng, 2021. The role of oxidative stress and antioxidants in diabetic wound healing. Oxid. Med. Cell. Longevity, Vol. 2021. 10.1155/2021/8852759.
25. Polaka, S., P. Katare, B. Pawar, N. Vasdev and T. Gupta *et al*., 2022. Emerging ROS-modulating technologies for augmentation of the wound healing process. ACS Omega, 7: 30657-30672.
26. de Souza Farias, S.A., K.S. da Costa and J.B.L. Martins, 2021. Analysis of conformational, structural, magnetic, and electronic properties related to antioxidant activity: Revisiting flavan, anthocyanidin, flavanone, flavonol, isoflavone, flavone, and flavan-3-ol. ACS Omega, 6: 8908-8918.
27. Raziyeva, K., Y. Kim, Z. Zharkinbekov, K. Kassymbek, S. Jimi and A. Saparov, 2021. Immunology of acute and chronic wound healing. Biomolecules, Vol. 11. 10.3390/biom11050700.
28. Rabkin, S.W., 2017. The Role Matrix Metalloproteinases in the Production of Aortic Aneurysm. In: Progress in Molecular Biology and Translational Science, Khalil, A. (Ed.), Elsevier, Netherlands, pp: 239-265.
29. Wan, S., S. Wan, X. Jiao, H. Cao and Y. Gu *et al*., 2021. Advances in understanding the innate immune associated diabetic kidney disease. FASEB J., Vol. 35. 10.1096/fj.202002334R.
30. Wu, T.C., S.T. Chan, C.N. Chang, P.S. Yu, C.H. Chuang and S.L. Yeh, 2018. Quercetin and chrysin inhibit nickel-induced invasion and migration by downregulation of TLR4/NF-κB signaling in A549 cells. Chem. Biol. Interact., 292: 101-109.
31. Spampinato, S.F., G.I. Caruso, R. de Pasquale, M.A. Sortino and S. Merlo, 2020. The treatment of impaired wound healing in diabetes: Looking among old drugs. Pharmaceuticals, Vol. 13. 10.3390/ph13040060.
32. Xue, M. and C.J. Jackson, 2015. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv. Wound Care, 4: 119-136.
33. Moghadam, E.R., H.L. Ang, S.E. Asnaf, A. Zabolian and H. Saleki *et al*., 2020. Broad-spectrum preclinical antitumor activity of chrysin: Current trends and future perspectives. Biomolecules, Vol. 10. 10.3390/biom10101374.