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Natural cocoa ingestion promotes wound healing in rats induced with diabetes by accelerating re-epithelialization and enhanced IGF-1 expression

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Abstract

Background: Diabetic wounds (DWs) are difficult to manage due to delayed healing, increasing the risk of infection and limb amputation. Hyperglycemia impairs re-epithelialization and dermal cell proliferation, which are key processes for wound closure. The persistent production of advanced glycation end products (AGEs) further inhibits healing in diabetes. Cocoa is rich in polyphenols, offers antioxidants, anti-inflammatory, and anti-glycemic properties that may support DW healing.

Aim: This study investigated the potential of natural cocoa powder to enhance wound healing in experimentally STZ-nicotinamide- induced type 2 diabetes (T2DM) rats.

Methods: T2DM was induced in rats using nicotinamide and streptozotocin. Rats with diabetes were assigned into a cocoa-treated group (DC) and an untreated group (DU); rats without diabetes served as controls (C). Aqueous 2% natural cocoa was administered to the DC group for 6 weeks. Full-thickness dorsal wounds were created, and biopsies were taken on days 0, 3, 7, and 14. Wound contraction, epidermal thickness, and dermal cell counts were assessed histologically. IGF-1 expression was also evaluated via immunohistochemistry.

Results: Cocoa-treated rats showed significantly enhanced wound contraction, thicker epidermis, and higher dermal cell counts. IGF-1 expression was markedly increased, comparable to levels in controls group.

Conclusion: Cocoa ingestion improves diabetes-induced wound healing, supporting its therapeutic potential.

Keywords: Diabetes-induced wounds, natural cocoa, IGF-1 expression, hyperglycemia, re-epithelialization

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by persistent hyperglycemia, and has an alarmingly growing global economic burden (1,2). The World Health Organization (WHO) estimates that about 830 million people worldwide live with diabetes, with the most cases occurring in low and middle-income countries (LMICs) (2). A large proportion of cases remain undiagnosed, resulting in delayed management and increased risk of complications. Africa and Asia are experiencing the fastest rise in diabetes prevalence (2), with Africa having the highest proportion of the undiagnosed population (3) in contrast with a lower rate in developed countries. Type 2 DM (T2DM) is the most common type of diabetes, accounting for 90-95% of all diabetes and is characterized by insulin resistance or inadequate production of insulin. Notably, 60% of Africa's adult population lives with undiagnosed

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T2DM compared to 38% in North America and the Caribbean region (3). Approximately 2.5% - 15% of annual global health budgets are spent on DM, with a greater proportion devoted to diabetes wound care (4). The management of T2DM complications, especially foot ulcer treatment, often requires long-term hospitalization and frequent outpatient visits with a burdensome sociocultural impact. Loss of mobility due to severe chronic foot ulcers imposes a great burden on the patient (such as stigma, loss of social status, social exclusion, and unemployment), family, and the healthcare system (5). Approximately 20% of T2DM individuals suffer from diabetic wounds with concomitant delayed healing, leading to wound infection and limb amputation (4).

Wound healing is a complex process involving overlapping phases of inflammation, proliferation, and remodeling (4). Disruption of any phase in this multistage process may delay wound healing. Ineffective re-epithelialization is an indicator of delayed wound healing that fails to proceed through the normal phases of orderly and timely healing, leading to chronic wounds (7). In DM, poor wound healing results from sustained inflammation and impaired cell proliferation, leading to the development of chronic ulcers. Poor management of T2DM causes various macro- and microvascular complications, including cardiomyopathy, encephalopathy, neuropathy, nephropathy, retinopathy, and foot ulcers (8,9, 10).

DM is characterized by significantly elevated levels of advanced glycation end-products (AGEs), which contribute to impaired wound healing (11,12). Considering that angiogenesis is crucial for initiating tissue growth and cell proliferation during wound healing, sustained inflammation and impaired angiogenesis are major contributors to the complex chemical mechanisms that underpin delayed wound healing in DM (9). Hyperglycaemia and hyperlipidemia are also known to inhibit normal neovascularization, causing a delay in diabetic wound (DW) healing (12,13). Moreover, impaired healing of DW is associated with sustained production of pro-inflammatory cytokines (14), impaired production of healing-associated growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF) 1 and 2; all of which delay DW healing (15). IGF-1 expression is upregulated in injured skin despite low baseline levels in the dermal and granular layers of normal skin (16). IGF-1 stimulates keratinocyte and fibroblast proliferation, inhibits apoptosis through suppression of inflammatory cytokines, and enhances extracellular matrix synthesis during granulation tissue formation (16). Reduced IGF-1 expression in diabetes has been implicated in delayed wound healing (15).

Cocoa is rich in flavonoids and natural compounds found in fruits, vegetables, and other plant products, and has antioxidant properties (17,18). Cocoa powder contains the highest variety of flavanols, a subclass of flavonoids that acutely increase skin microcirculation (19–21). Antioxidants are known to protect cells against damage from free radicals and are essential for tissue granulation during wound healing (22). It has been speculated that the beneficial antioxidant effects of cocoa and its ability to promote vascularization may be important for the wound healing process (23). Prandial consumption of cocoa or chocolate has been shown to enhance vascular function through nitric oxide–mediated endothelial activity, improve insulin sensitivity and inflammatory responses (21,23). These effects may be important in cellular proliferation and may enhance healing of diabetes-induced wounds by scavenging free radicals generated as a result of impaired glucose metabolism in diabetes (24).

The present study investigated the potential of natural cocoa powder to improve wound healing in rats with experimentally induced T2DM. Cocoa flavanols enhance IGF-1 signaling by reducing oxidative stress and improving endothelial function, thereby promoting keratinocyte proliferation and migration essential for re-epithelialization in diabetic wounds (7, 9). In addition, cocoa polyphenols upregulate IGF-1 expression and downstream pathways such as PI3K/Akt signaling pathway, facilitating angiogenesis and tissue regeneration that accelerate wound closure under hyperglycemic conditions (4, 25).

MATERIALS AND METHODS

Ethical Considerations

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Ethical and Protocol Review Committee (EPRC) of the University of Ghana College of Health Sciences, under approval number CHS-Et/M.6-P4.6/2021-2022. The study also adhered to the ethical principles outlined in the Guide for the Care and Use of Laboratory Animals and complied with national regulations governing animal research. Every effort was made to minimize animal suffering, reduce the number of animals used, and apply humane endpoints throughout the study.

Study site and conditions

This study was conducted at the Animal Experimentation Unit of the University of Ghana Medical School, Korle Bu. The experimental room had a temperature of 25±2º C and a humidity of 60±4%.

Study Design and Approach

The study involved 3 groups of adult male Sprague Dawley rats (15-17 weeks old) weighing between 210 g and 310 g.

Cocoa Drink Preparation (2% w/v)

A 2% weight per volume (w/v) concentration of unsweetened natural cocoa (GoodFood® brand, Ghana: polyphenols and theobromine as bioactive minerals) drink

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was prepared daily as previously described (19) and administered to the rats in the DC group for the entire 6-week duration of experimentation. The freshly prepared suspension was divided into six portions of 100 ml aliquots for each rat. Each rat was given 100 ml of natural cocoa drink dispensed in a 200 ml graduated feeding bottle and suspended in each cage for 12 hours. This was alternated with 12 hours of regular drinking water. The suspension was shaken every 2 hours to ensure a homogenous solution. The feeding bottles were washed every day to prevent microbial growth.

Induction of T2DM

Rats were acquired from the animal house of Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, and allowed to acclimatize for 1 week. Rats were housed individually in cages, each measuring 60 mm by 80 mm and suspended over sawdust bedding to receive animal dung and urine. They were fed standard rat chow and water *ad libitum*. The rats were fasted overnight (8-10 hours) but were allowed free access to drinking water to prevent dehydration, after which baseline blood glucose levels were determined from a tail vein sample collection. Sixteen (16) rats (8 each in the DC and DU groups) were injected intraperitoneally (IP) with 110 mg/kg nicotinamide (NCTD) (Glentham, GLS GP9253-25G, UK) dissolved in normal saline (N/S) at a concentration of 110 mg/ml. This was followed, 15 minutes later, by a single dose of 55 mg/kg Streptozotocin (STZ) (Glentham, GLS GA1956-1G, UK). A breakthrough diet of 5% glucose solution with standard rat chow was given to all the rats overnight to prevent hypoglycemic shock, after which the rats resumed regular assigned diet. Fasting blood glucose (FBG) was measured 72 hours after NCTD/STZ administration and repeated on day 7 following NCTD/STZ administration to confirm T2DM at a fasting blood glucose (FBG) level of ≥7.5 mmol/L.

Experimental and control groups

Diabetes-induced rats were randomly assigned into two (2) groups of eight (8) each. The diabetes group treated with cocoa (DC) was treated with voluntary ingestion of a natural cocoa suspension for 6 weeks. The second group (diabetes untreated, DU) did not receive the natural cocoa intervention. Two (2) rats from DU group died, one on days 8 and another on 14 after diabetes induction. Six (6) rats from both DC and DU were therefore included in the experimental groups. Four (4) rats without diabetes (C) comprised the third group, which served as controls.

Creation of wounds and tissue biopsies

Four (4) weeks after the start of the experiment, the fasting blood glucose (FBG) levels of all groups were determined to confirm hyperglycemia in DC and DU, and normoglycemia in C rats. A surgical wound was created on the dorsal surface of the lumbosacral region of all the rats as follows. The rats were anaesthetized by intraperitoneal injection of 75 mg/kg ketamine and 5 mg/kg xylazine. Next, the dorsal surface was shaved and disinfected with 70% ethanol before a full-thickness skin excision, measuring approximately 2 cm × 2 cm (Fig. 1A). The wound was created with a size 11" surgical blade. The wounds were irrigated with normal saline and left uncovered to avoid entanglement of gauze with the regrowing hair and to minimize disruption of granulation tissue. All rats received identical wound management with respect to dressing.

Wound margin biopsy (5 mm × 5 mm) was performed on days 0, 3, 7, and 14, with alternating margins starting from the margin towards the (cranial) to the right lateral, caudal, and left lateral margins (Fig. 1B and 1C). On days 0 and 14, cranial and caudal biopsies were obtained from all 16 rats. On days 3 and 7, three (3) rats each from DC and DU groups and two (2) rats from C group were selected for right and left lateral biopsies, respectively. Biopsies were taken alternatively from different rats on different days to allow for adequate procedural recovery, avoid repeated stress induced by anesthesia, and to minimize interference with healing at the wound site.

Measurement of glycated haemoglobin (HbA1c)

On day 14 post-wounding, after the last biopsy was taken from the rats in all groups, a cardiac puncture was performed using a 21-gauge needle to withdraw blood for percentage glycated hemoglobin analysis. Approximately 2 ml blood was collected into an EDTA specimen container before tissue perfusion was performed to euthanize the animals. The blood specimens were immediately sent to the laboratory for analysis using a Vitros 4600 Chemical Analyzer (Ortho Clinical Diagnostics, USA). Analysis of the samples was carried out by laboratory personnel who were blinded to treatment assignments.

Percentage Wound Contraction Estimation

The percentage of wound contraction was estimated by objectively measuring the area of the wound. A Vernier caliper was used to measure the wound area on days 0, 3, 7, and 14 in all groups when the animals were under anesthesia and before skin biopsies were taken. The percentage of wound contraction was calculated using Wilson's formula as follows:

$$\%\ Wound\ Contraction = \left(\frac{Ao - Ay}{Ao}\right) x\ 100$$

Where *Ao* is wound area at Day 0, and *Ay* is wound area at Day Y (days 3, 7, and 14).

Tissue processing

A full-thickness skin biopsy (5 mm × 5 mm) taken from each wound margin was immediately fixed by immersion in 10% neutral buffered formaldehyde for 24 hours. Fixed tissues were dehydrated with graded ethanol (50%, 70%, 90%, 95%, and 100%) and embedded in paraffin wax after clearing with xylene. Using a Leica microtome (Leica RM 2125), the blocked tissues were trimmed to completely expose them. The tissue was then sectioned at a thickness

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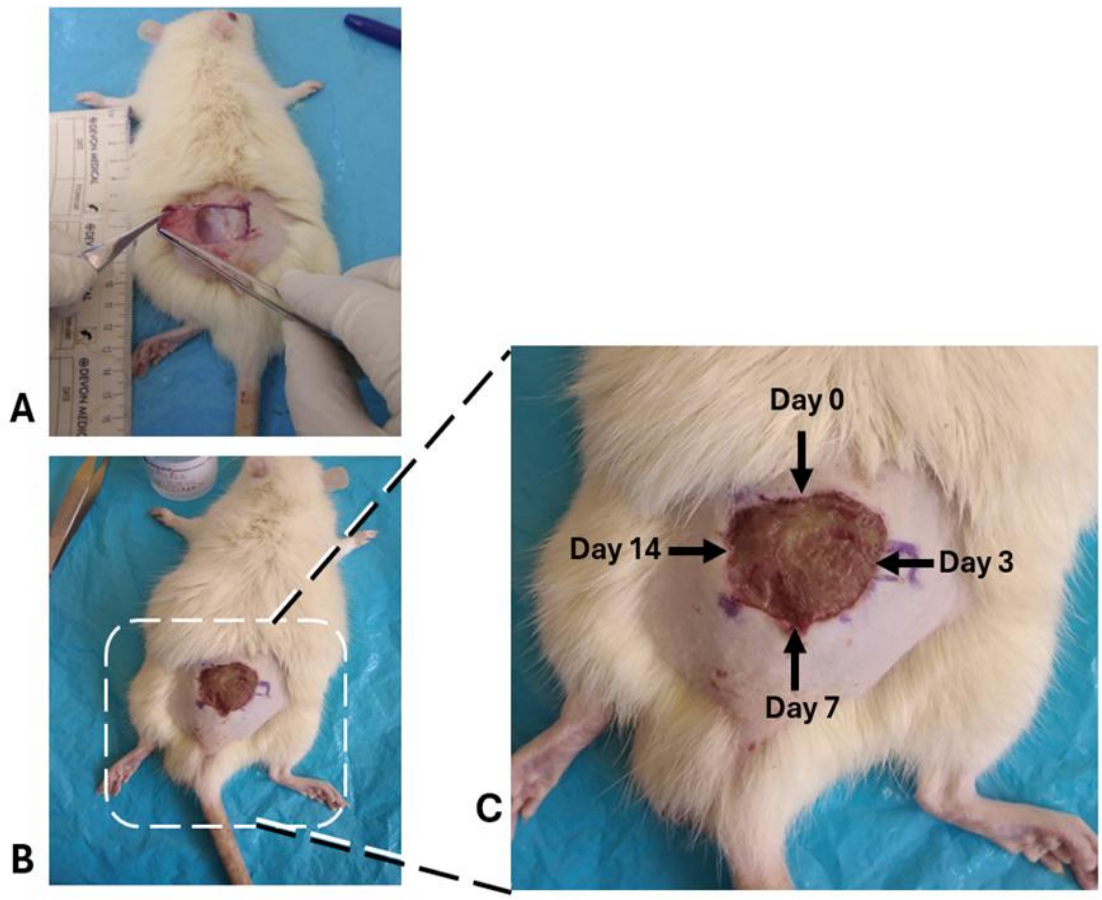

Figure 1. A picture of a rat with a wound created at the lumbosacral region on Day 0 (A) and the positions of tissue biopsies (B and C) on Days 3, 7, and 14.

of 5 µm. Three sections were systematically selected 15 µm apart from each biopsy, stained with hematoxylin and eosin (H&E), mounted using dibutyl phthalate polystyrene xylene (DPX), and covered with coverslips.

Epidermal thickness, Reticular Cell Count and Fibroblasts Population Evaluation

A S-EYE MC- D200U (Shenzhen Hayear Electronics Co. Ltd, China) camera measuring tool was used to determine the epidermal thickness on tissue slides using the x4 (objective lens). The pixel lengths were converted to microns by calibration using a stage graticule with 1.0 mm graduations. At the same magnification, 1.0 mm of the stage graticule corresponded to 400.89-pixel length (1 pixel = 2.49 µm). Image J© software version 1.46r (Broken Symmetry, UK) was used to quantify the fibroblasts and cells in the reticular layer of the dermis using point counting stereology. Reticular dermal cells were identified in the micrographs using their purple nuclei. All nuclei within the dense, irregular connective tissue in the reticular layer of the dermis were counted. Fibroblasts were distinguished by their purple-stained, slender nuclei.

IGF-1 Protein Expression Level Estimation

Two tissue sections (7th and 14th) per biopsy block from days 7 and 14 from the three (3) groups were systematically sampled. Twelve (4 × 3) sections were prepared for IHC staining. The paraffin-embedded sections were heated in an oven at a temperature of 40°C for 12 hours, de-waxed in two changes of xylene for 5 min each, and rehydrated in graded alcohols, and immersed in citrate buffer. Antigen retrieval was performed by boiling the sections in 10 mM citrate buffer (pH 7.4) in a pressure cooker for 15 min at 120°C and washing with 0.01 M phosphate-buffered saline (PBS; pH 7.4) three times for 5 min each. Sections were blocked with 1% bovine serum albumin for 10 min.

After washing, endogenous peroxidase activity was removed by incubating sections in 3% hydrogen peroxide for 5 minutes and then washing with citrate buffer. The sections were incubated with an anti-IGF-1 antibody (Biocam, USA) at room temperature for 1 hour at a

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dilution of 1:500. The sections were incubated with goat anti-rabbit antibody (Horse Radish Peroxidase/HRP) at a 1:500 dilution for 45 minutes. The color reaction was carried out using a diaminobenzidine (DAB) (Abcam/USA) substrate for 5 minutes.

Hematoxylin (0.2%) was used to counterstain cell nuclei. The IHC staining intensity of IGF-1 in the epidermis and dermis at the wound border, 5 mm from the granulation tissue, was assessed. Assessment was performed microscopically by two independent assessors. The mean staining intensity was assessed semi-quantitatively as follows: 0 = no staining (-), 1= weak staining (+/-), 2 = moderate staining (+), 3 = strong staining (++), and 4 = very strong staining (+++). The mean value of the two scores from all the sections was averaged as the value for IGF-1 expression in the epidermis and dermis.

Data Analysis

Data was entered into GraphPad Prism version 8.0 for analysis. The mean values of weekly weight, fasting blood glucose (FBG), daily fluid intake, epidermal thickness, percentage wound contraction, and reticular cell population were compared between groups using one-way ANOVA, followed by Tukey's post hoc test to compare the means within the groups. A paired t-test was used to compare the variables of rats before and after DM induction. Non-parametric variables were analyzed using the Kruskal–Wallis' test. The results were presented as the mean ± standard error (SE) of the mean. The significance level for rejecting the null hypothesis was set at 5%.

RESULTS

Fasting blood sugar levels

The mean baseline fasting blood glucose (FBG) of the rats selected for DM induction was 5.042 mmol/L (SE = 0.475). In comparison, the baseline mean FBS of the control group was 5.000 mmol/L (SE = 0.636) with no significant difference (t = 1.603, p = 0.1699) between the 2 groups before DM induction. Seven days after DM induction, the FBS levels of rats with cocoa-treated diabetes (DC), rats with cocoa-untreated diabetes (DU), and rats without diabetes [control (C)] groups were 25. 21 mmol/L (SE = 6.371), 18.72 mmol/L (SE = 8.599), and 4.900 mmol/L (SE = 0.790), respectively, with notable significant differences between the groups (t = 8.603, p = 0.0004) (Fig. 2A). The mean FBS level post-induction of diabetes in the DC group gradually decreased from weeks 2 to 3 and increased only in week 4, after which there was a gradual decrease again from weeks 4 to 7 (Fig. 2A). The FBS in the group with diabetes without cocoa (DU), on the other hand, increased from weeks 2 to 4, after which a steady decrease was observed from week 5 to week 7 in a pattern similar to that of the DC group. The experimental control group (C) recorded the lowest FBS, which was almost constant throughout the 6-week experimental period.

Percentage glycated haemoglobin (HbA1c)

The mean percentages glycated haemoglobin (HbA1c) in DC, DU, and C groups was 5.128% (SE = 0.717), 5.157 (SE = 0.547), and 3.632% (SE = 0.193), respectively. Although there was a significant difference between DC and C (p = 0.0014) and DU and C (p = 0.0012), there was no statistically significant difference between the two (2) groups with diabetes (DC and DU) (t = 0.9401; p = 0.07) (Fig. 2B).

Epidermal Thickness and Reticular Cell Count

The groups with diabetes (DC and DU) had thinner epidermal thickness from day 0 to day 14 than the control group (Fig. 3A). The epidermal thickness within the DC group increased significantly from day 0 to day 3 (p =0.0459), marginally decreased from day 3 to day 7 (p =0.8368), and subsequently increased significantly until day 14 (p = 0.0150). The same pattern was recorded in DU, but no significant difference was recorded between days within the group (Fig. 3A). In the control group (C), epidermal thickness increased from day 0 to day 3 (p =0.2952), with a significant difference between days 0 and 7 (p = 0.0485), followed by a significant decrease from day 14 to day 0 (p = 0.0042).

The cell population in the reticular dermis showed no significant differences between or within the groups (Fig. 3B). However, the groups with diabetes had the lowest cell count on day 0, although the DC group had a higher count than the DC group. Both the DC and DU groups had elevated cell counts on day 3, a non-significant decrease on day 7, and an increase on day 14 (Fig. 3B). The control group had the highest cell count on day 0, which decreased on day 3, increased on day 7, and again decreased on day 14.

Percentage wound contraction

The DC and C groups showed comparable wound contraction, which was significantly greater than that of the DU group (Fig. 4; Table 1). There was a significant difference within each group, with the DC group recording a significantly higher percentage of wound contraction than the DU group (p = 0.0007 and p = 0.035, respectively). A significant difference in the mean percentage of wound contraction was observed on days 3 and 14 in the groups with diabetes (DC and DU), but not in the control group. In all groups, there was a significant difference between days 7 and 14 (p = 0.0066, 0.0124, and 0.0054, respectively) (Table 1).

IGF-1 Expression in Epidermis and Dermis of Skin

The mean expression of IGF-1 protein in the epidermis between the groups on day 14 was significantly different (p=0.009), though there was no significant difference in the mean expression in the epidermis on day 7 between the groups (Table 1). The mean expression in the epidermis on day 7 was moderate (+) in both DC and C, while DU showed weak expression (+/-). On day 14, the mean expression in the epidermis was very strong (+++) in C, strong (++) in DC, and moderate (+) in DU.

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The mean IGF-1 protein expression in the epidermis in DC was higher than that in DU, but not statistically significant on days 7 and 14 (p = 0.1711 and 0.1894, respectively). However, the mean IGF-1 protein expression on day 14 was significantly lower in the DU group than that in the C group (p<0.005).

No significant difference was observed in IGF-1 protein expression in the dermis on days 7 and 14 (Table 1) between the groups. On day 7, the mean protein expression in the dermis in both DC and DU was weak (+/-), whereas on day 14, the expression remained weak (+/-) in DU but moderate (+) in both DC and C groups (Table 1 and Fig. 5).

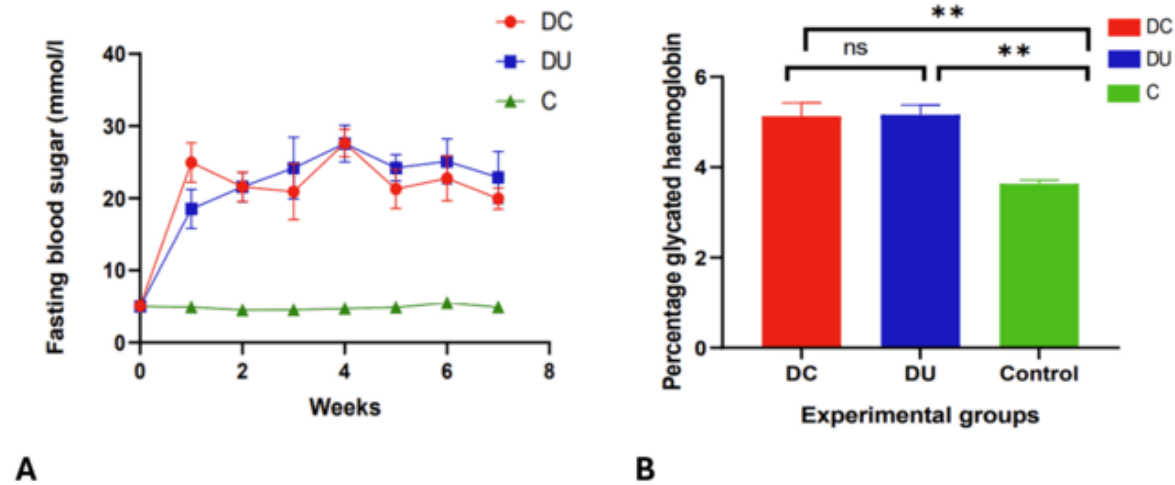

*Figure 2: Graphs illustrating the weekly changes of plasma FBS (**A**) and percentage glycated haemoglobin (HbA1c) (**B**) in the rat groups. The error bars represent the standard error of the mean. DC = group with diabetes with Cocoa, DU = group with diabetes Untreated (without cocoa), C = Control group. ns= non-significant; **p≤ 0.005*

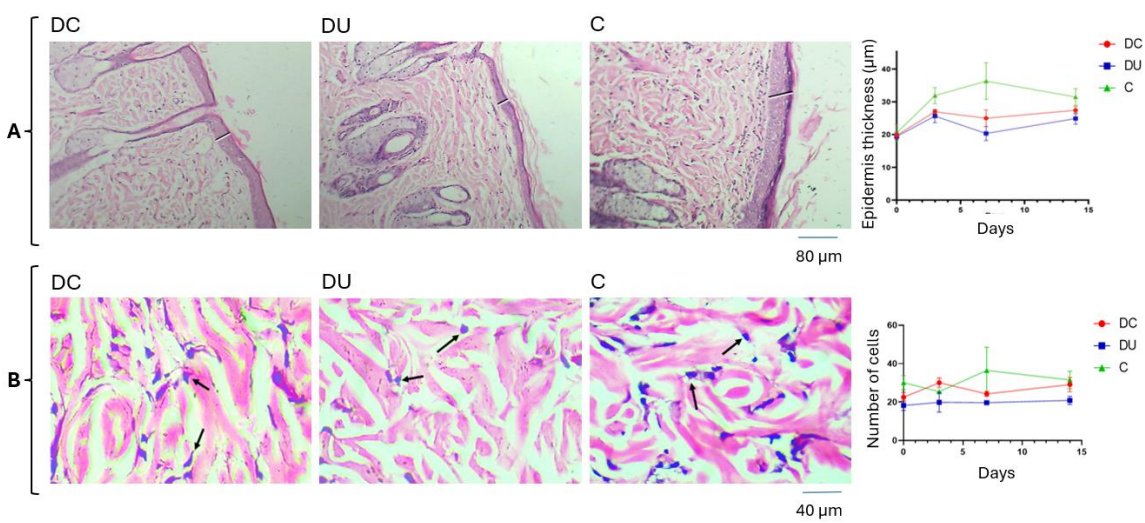

Figure 3: Representative micrographs of skin tissue demonstrating epidermal thickness (Panel A) and cell proliferation (Panel B) on day 14 with respective line graphs on days 0, 3, 7 & 14 in the three rat groups. The error bars represent the standard error of the mean in the experimental rat groups. DC = Diabetic with Cocoa, DU = Diabetic Untreated (without cocoa), C = Control group.

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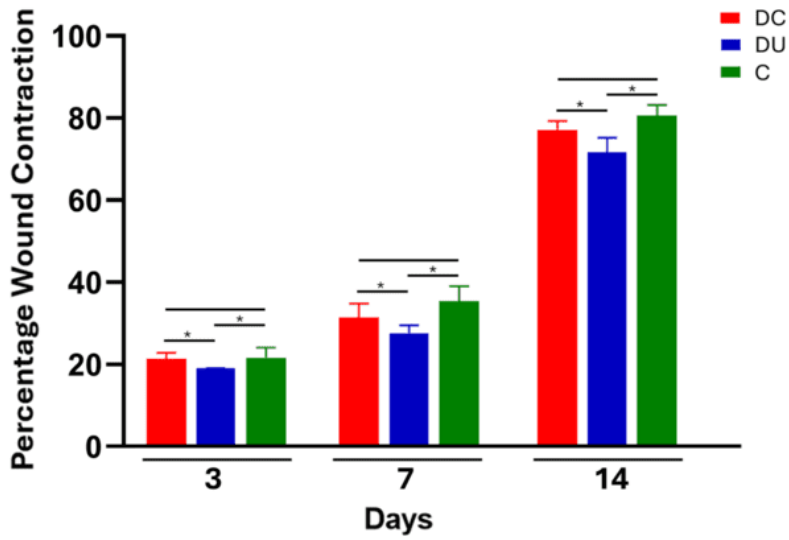

Figure 4: Percentage wound contraction on days 3, 7 & 14 in the three rat groups. DC = group with diabetes with Cocoa, DU = group with diabetes Untreated (without cocoa), C = Control group.

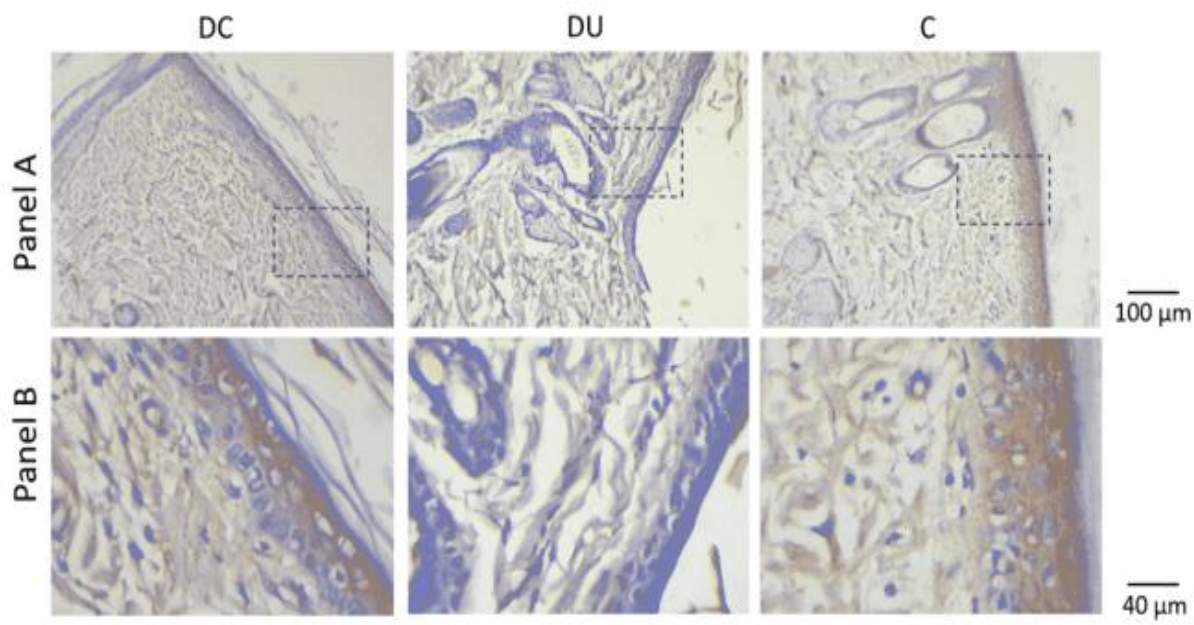

Figure 5: Immunohistochemistry staining of IGF-1 from Day 14 tissue sections. Panel A has images taken at x10. Panel B has images of magnified ringed sections of Panel A. DC = group with diabetes with Cocoa, DU = group with diabetes Untreated (without cocoa), and C = Control group.

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Table 1: Summary of percentage wound contraction and IGF-1 protein expression

Wound contraction				
	Mean Percentage Wound Contraction (SD)			
Day	DC (*n=6*)	DU (*n=6*)	C (*n=4*)	p-value 1
3	23.99 (2.86)[a] **	21.43 (0.33)[a]	24.86 (3.96)	
7	35.07 (6.46)[b] **	30.02 (3.50)[b]*	39.39 (7.09)[b]**	0.035
14	86.19 (5.53)	80.57 (10.02)	88.31 (6.03)	0.026
p-value 2	0.0007(269.80)	0.0134 (94.840)	0.0035 (201.30)	0.025

IGF-1 protein expression			
	IGF-1 Staining Intensity (SD)		
Day	DC (*n=6*)	DU (*n=6*)	C (*n=4*)
Epidermis			
7	+	+/-	+
14	++	+	+++
Dermis			
7	+/-	+/-	+
14	+	+/-	+

Wound Contraction: A p-value of 1 represents the probability of a statistical relationship between DC, DU, and C using one-way ANOVA with Tukey's post hoc comparison. The p-value 2 represents the statistical relationship within each group over time using a repeated-measure ANOVA. a - Statistical difference between days 3 and 14, and b - statistical difference between days 7 and 14. *p-value ≤ 0.05; ** p-value ≤ 0.005

IGF-1 protein expression: Kruskal-Wallis test with Dunn's multiple comparison post hoc test between DC, DU, and C and Wilcoxon matched-pairs test between two groups; p-value 1 represents the probability of a significant difference between the groups; p-value 2 represents the probability of a significant difference within the groups; $= Statistical difference between DU and C; a= p≤0.005; DC= diabetes with cocoa, DU= diabetes without cocoa, C= control, 0= no staining (-), 1= weak staining (+/-), 2= moderate staining (+), 3=strong staining (++), and 4= very strong staining (+++).

DISCUSSION

In the present study, diabetic rats receiving cocoa (DC) exhibited lower fasting blood glucose (FBG) levels compared to untreated diabetic rats (DU), except in week 1. However, both diabetic groups showed comparable HbA1c percentages, likely due to the short duration of the study. Fernández-Millán et al. (25) demonstrated that cocoa consumption improves glycemic control, insulin sensitivity, and preserves pancreatic β-cell mass. Although cocoa flavanols are known to enhance glucose homeostasis by reducing carbohydrate digestion and absorption (24), the expected improvement in HbA1c was not observed, possibly due to the limited study duration. Although glycated haemoglobin (HbA1c) is classically used as a long-term marker of glycaemic control in humans, its assessment in rodents is justified by the shorter lifespan of rat erythrocytes (approximately 45–65 days), allowing HbA1c to reflect cumulative glycaemic exposure over a 4–6-week period. In the present study, HbA1c was included as a complementary metabolic index to fasting blood glucose to distinguish transient hyperglycaemia from sustained glycaemic exposure.

Consistent with existing evidence, diabetic rats showed elevated FBS and HbA1c compared to controls, reflecting increased glycation associated with sustained hyperglycemia (26,27). This process contributes to impaired protein function and delayed wound healing by reducing the activity of key cells such as keratinocytes, fibroblasts, macrophages, and endothelial cells, as well as diminishing growth factor production, including IGF-1 (4,9,29). Although HbA1c was not significantly reduced, the lower FBS in DC rats suggests improved short-term glycemic control, likely due to cocoa's bioactive components, including polyphenols, magnesium, chromium, and theobromine (18,24).

Wound healing outcomes were improved in cocoa-treated diabetic rats, as evidenced by increased wound contraction, dermal cell count, epidermal thickness, and IGF-1 expression (25,31). While diabetic groups exhibited reduced epidermal thickness compared to controls, the DC group showed recovery from day 7 onward, indicating enhanced keratinocyte proliferation and re-epithelialization. In contrast, untreated diabetic rats demonstrated early epidermal thinning, suggesting delayed cell migration and proliferation (33). The improvement in the DC group may be attributed to cocoa's antioxidant and anti-inflammatory properties (25,31), which support progression from the inflammatory to the proliferative phase of healing (32). Increased dermal cell counts, particularly fibroblasts, between days 7 and 14 indicate enhanced granulation tissue formation and collagen synthesis (37). Similar findings have been reported in treated diabetic wounds, showing increased fibroblast activity and vascularization.

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Growth factor expression, particularly IGF-1, is suspected to have played a central role in these outcomes. IGF-1 levels in DC rats were comparable to controls and higher than in DU rats, supporting enhanced cell proliferation, fibroblast activity, and wound contraction (4,16,38). Given that diabetes is associated with reduced growth factor expression (39), the relative preservation of IGF-1 in cocoa-treated rats likely contributed to improved healing, including re-epithelialization and tissue remodeling. Cocoa bioactives, especially flavonoids and polyphenols, modulate growth factor pathways such as IGF-1, VEGF, and TGF-β (20). In this study, the enhanced wound contraction observed in DC rats, particularly between days 7 and 14, suggests an accelerated proliferative phase. This effect is likely mediated by improved cellular activity and growth factor signaling rather than by long-term glycemic changes alone (18,23,25).

Dietary inclusion of 2% cocoa in rodent chow is commonly used in experimental models, and when adjusted for differences in energy intake and body surface area, approximates a moderate-to-high, but achievable human intake of cocoa flavanols, roughly equivalent to regular consumption of flavanol-rich cocoa or dark chocolate products (31). However, depending on the flavanol concentration of the cocoa used, this dose may verge toward a supra-physiological exposure relative to typical human diets, especially in populations with low habitual cocoa intake (25).

In this present study, cocoa supplementation improved short-term glycemic control and enhanced wound healing in diabetic rats through modulation of cellular proliferation and growth factor expression. However, the study duration was insufficient to demonstrate changes in HbA1c, and no mechanistic study was conducted to fully elucidate the pathways by which cocoa influences IGF-1 and related processes.

Conclusion

The current study investigated the effect of prandial cocoa ingestion on wound healing in rats with experimentally induced T2MD. Consumption of unsweetened natural cocoa corresponded with a lower glycaemic index. Increased percentage wound contraction, epithelialization, fibroblast population, and IGF-1 protein expression were observed in diabetic rats treated with cocoa. The dietary nutrients of cocoa upregulated IGF-1 protein expression, promoted dermal cell proliferation, and enhanced epidermal re-epithelialization in diabetes-induced rats, thereby promoting wound healing.

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Declaration of Interest

The authors declare that they have no potential competing interests, except for Frederick Kwaku Addai (FKA). FKA is a non-salaried Director of KEL Kakawa Company Ltd., which packages GoodFood® natural Cocoa Powder for health-promoting consumption in Ghana. KEL made no financial contribution to this study, which was an MPhil. thesis project for which FKA was part of the supervisory team.

Data Availability

All supporting data have been included in the manuscript. Source files are available upon request from the corresponding author.

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