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Biochanin A Alleviates Oxidative Stress in Diabetic Rat Skin

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ABSTRACT

Background: Diabetes mellitus is a significant global health issue, frequently leading to skin complications exacerbated by chronic hyperglycemia and associated oxidative stress. Oxidative stress, characterized by increased reactive oxygen species (ROS), reduced antioxidant defenses (e.g., Catalase - CAT), and elevated lipid peroxidation (e.g., Malondialdehyde - MDA), plays a critical role in impairing skin integrity in diabetic individuals. The Nrf2/ARE pathway is a key regulator of cellular antioxidant responses. Biochanin A (BCA), a natural isoflavone, possesses known antioxidant properties. This study aimed to evaluate the efficacy of topically applied Biochanin A in mitigating oxidative stress within the skin tissue of streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was induced in male Wistar rats using STZ (50 mg/kg, I.P.). Diabetic rats were randomly assigned to receive topical treatment with either a BCA-loaded hydrogel (5% BCA in 1.5% HPMC), vehicle hydrogel (1.5% HPMC), or no treatment for 14 days. A non-diabetic control group was also included. Following the treatment period, skin tissue surrounding a surgical cut was collected. Oxidative stress markers were assessed by measuring MDA levels and CAT activity. The expression of Nrf2 and its downstream target NQO1 was evaluated using immunohistochemistry. Statistical analysis was performed using ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Results: Compared to the normal control group, untreated diabetic rats showed significantly increased MDA levels and significantly decreased CAT activity, Nrf2 expression, and NQO1 expression in skin tissue. Treatment with BCA significantly reduced MDA levels and significantly increased CAT activity compared to both untreated diabetic and vehicle-treated groups. Furthermore, BCA treatment significantly upregulated the expression of Nrf2 and NQO1 compared to untreated diabetic and vehicle-treated groups. The vehicle group showed minimal effects compared to the untreated diabetic group.

Conclusion: Topical application of Biochanin A effectively mitigates oxidative stress in the skin of diabetic rats. This protective effect appears to be mediated through the enhancement of antioxidant enzyme activity (CAT), reduction of lipid peroxidation (MDA), and activation of the Nrf2/NQO1 signaling pathway. These findings suggest that BCA holds therapeutic potential for managing oxidative stress-associated skin complications in diabetes.

Keywords: *Biochanin A, Diabetes Mellitus, Oxidative Stress, Nrf2 Pathway, Skin*

1. NTRODUCTION

Diabetes mellitus stands as one of today's most pressing global health challenges, affecting more than half a billion people worldwide, with over 10.5% of the adult population living with this condition. What makes diabetes particularly complex is not just its increasing prevalence, but its wide-ranging effects on multiple body systems, especially the skin. The relationship between diabetes and dermatological conditions has become an area of significant clinical interest, as skin manifestations often serve as early indicators of the disease (1,2).

The World Health Organization provides a comprehensive understanding of diabetes mellitus, describing it as a multifaceted metabolic disorder with various underlying causes. At its foundation, the condition is characterized by chronic hyperglycemia, accompanied by significant disturbances in the body's processing of proteins, carbohydrates, and fats (1–4). These metabolic disruptions stem from either impaired insulin production, reduced insulin effectiveness, or a combination

of both factors (1,3).

The skin, being the body's largest organ, often reflects the internal metabolic changes associated with diabetes. Clinical observations reveal that between 30% and 70% of individuals with both Type 1 and Type 2 diabetes will develop some form of skin complication during their disease progression. These dermatological manifestations present across a broad spectrum, ranging from mild cosmetic concerns to severe, potentially life-threatening conditions (2,4,5) . Healthcare providers have identified several distinct categories of diabetes-related skin conditions. A diverse range of dermatological conditions can occur in patients with DM. Conditions with a strong association include acanthosis nigricans, diabetic dermopathy, diabetic foot issues, limited joint mobility, bullosis diabeticorum, scleredema diabeticorum, and necrobiosis lipoidica. Non-specific signs and symptoms frequently observed alongside DM encompass palmar erythema, rubeosis faciei diabeticorum, ichthyosis-like changes on the calves' integument, cutaneous xerosis, eruptive xanthomas, acrochordons, pruritus linked to diabetes, keratosis pilaris, pigmented purpuric dermatoses, yellow nail and skin syndrome, and onychocryptosis. Furthermore, specific dermatological disorders like generalized granuloma annulare, psoriasis, lichen planus, vitiligo, and hidradenitis suppurativa are connected to DM. Other relevant classifications involve secondary skin problems, heightened vulnerability to infections, and skin changes stemming from diabetes therapies(1,5,6).

Diabetes-related metabolic disorders lead to disruption in redox balance, triggering oxidative stress through an unbalanced relationship between reactive oxygen species (ROS) generation and elimination(7) . At the physiological level, when glucose levels rise, increased oxygen consumption leads to ROS formation, which acts as signaling molecules that trigger glucose-stimulated insulin secretion, a core function of pancreatic β-cells (8). However, chronic hyperglycemia enhances multiple pro-oxidative pathways, including the polyol pathway, formation of AGEs, PKC activation, and hexosamine pathway, collectively elevating ROS production and contributing to diabetic complications through persistent inflammation and cellular damage (9) Meanwhile, patients with diabetes exhibit a compromised antioxidant defense, as indicated by decreased levels of ROS-detoxifying enzymes, such as superoxide dismutase (SOD), catalase (CAT) (10). Malondialdehyde (MDA) serves as a critical biomarker for oxidative stress assessment, formed during lipid peroxidation of polyunsaturated fatty acids in cell membranes. Elevated MDA levels indicate increased oxidative damage and are consistently observed in diabetic skin, correlating with impaired healing outcomes (11).

Current research demonstrates that oxidative stress, particularly through excessive reactive oxygen species (ROS) production, plays a pivotal role in impaired skin healing processes, with this mechanism being especially critical in diabetic skin where the redox imbalance significantly delays tissue repair and regeneration (12,13).

The transcription factor Nrf2 (Nuclear factor erythroid 2-related factor 2) plays a crucial role in cellular defense mechanisms by orchestrating antioxidant responses through its interaction with specific DNA regions known as antioxidant-response elements (AREs) (14) .When the Nrf2/ARE pathway becomes activated, it enhances the production of various protective proteins that combat oxidative stress, including superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) (15).

Biochanin A, a naturally occurring O-methylated isoflavone (5,7-Dihydrox-4'-methoxyisoflavone), is found primarily in red clover and alfalfa plants (16). This bioactive compound has garnered significant attention for its diverse therapeutic properties across multiple biological systems. Research has demonstrated its effectiveness as an anticancer, hepato-protective, anti-inflammatory, antibacterial, antioxidant and neuroprotective properties (17). Therefore, in this study, we investigated whether BCA alleviates oxidative stress in the skin of diabetic rats.

2. MATERIALS AND METHODS

This study utilized male Wistar rats (190-230 g) obtained from the King Abdulaziz University (KAU) animal facility, housed under standard conditions, and the protocol was ethically approved. Diabetes was induced via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg; Sigma Aldrich) in citrate buffer (pH 4.5). Rats with blood glucose levels between 200-300 mg/dl after 7 days (Accu-check Instant glucometer) were included. Rats were randomly assigned (n=10/group) to: Normal Control (non-diabetic, untreated), Diabetic Control (diabetic, untreated), Vehicle (diabetic, treated with 1.5% Hydroxypropyl methyl cellulose [HPMC] hydrogel), and BCA (diabetic, treated with hydrogel containing 5% Biochanin A [BCA; Aktin Chemicals]). Hydrogels were prepared by dispersing HPMC (Sigma Aldrich) ± BCA in distilled water. Under ketamine/xylazine anesthesia, a 1 × 1 cm^2 full-thickness surgical cut was created on the dorsal skin. Treatments were applied topically around the incision site daily for 14 days. On day 15, rats were sacrificed, and skin tissue surrounding the incision site was collected. One portion was fixed in 10% neutral buffered formalin for histology and immunohistochemistry (IHC); the other was stored at -80°C for biochemical analysis. For biochemical assays, tissue homogenates (10% w/v in PBS) were prepared and centrifuged. Supernatants were used to measure malondialdehyde (MDA) and Catalase (CAT) activity using commercial colorimetric kits (Cayman Chemical) according to manufacturer protocols, with readings taken via spectrophotometry/plate reader. For IHC, formalin-fixed, paraffin-embedded sections (4 µm) were stained using primary monoclonal antibodies against Nrf2 and NQO1 (Abcam). Detection was performed using HRP/DAB detection kits

(Biotechne). Staining intensity was quantified using ImageJ software (v1.46a, NIH). Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism (v8.1). One-way ANOVA followed by Tukey's post hoc test was used for group comparisons. A p-value < 0.05 was considered statistically significant.

3. Result

Effect of BCA on antioxidative status

BCA decreases MDA concentration

In the present study, oxidative stress was assessed by measuring MDA levels in different experimental groups. The results demonstrated that the untreated diabetic group showed a significant accumulation of MDA (Figure 1A) compared to the control group. When the diabetic animals were treated with BCA or vehicle, the BCA-treated group exhibited a marked reduction in MDA levels compared to the untreated diabetic group. In contrast, the vehicle-treated group showed only minimal improvement in MDA levels.

BCA increases CAT activity

In the present study, antioxidant defense system was evaluated by measuring CAT activity in different experimental groups. The results revealed that the untreated diabetic group exhibited a significant decrease in CAT activity (Figure 1B) compared to the control group. When the diabetic animals were treated with BCA or vehicle, the BCA-treated group demonstrated a marked restoration in CAT activity compared to the untreated diabetic group. The CAT activity in the BCA-treated group was significantly higher than that observed in the vehicle-treated group. In contrast, the vehicle-treated group showed only modest improvement in CAT activity compared to the untreated diabetic group.

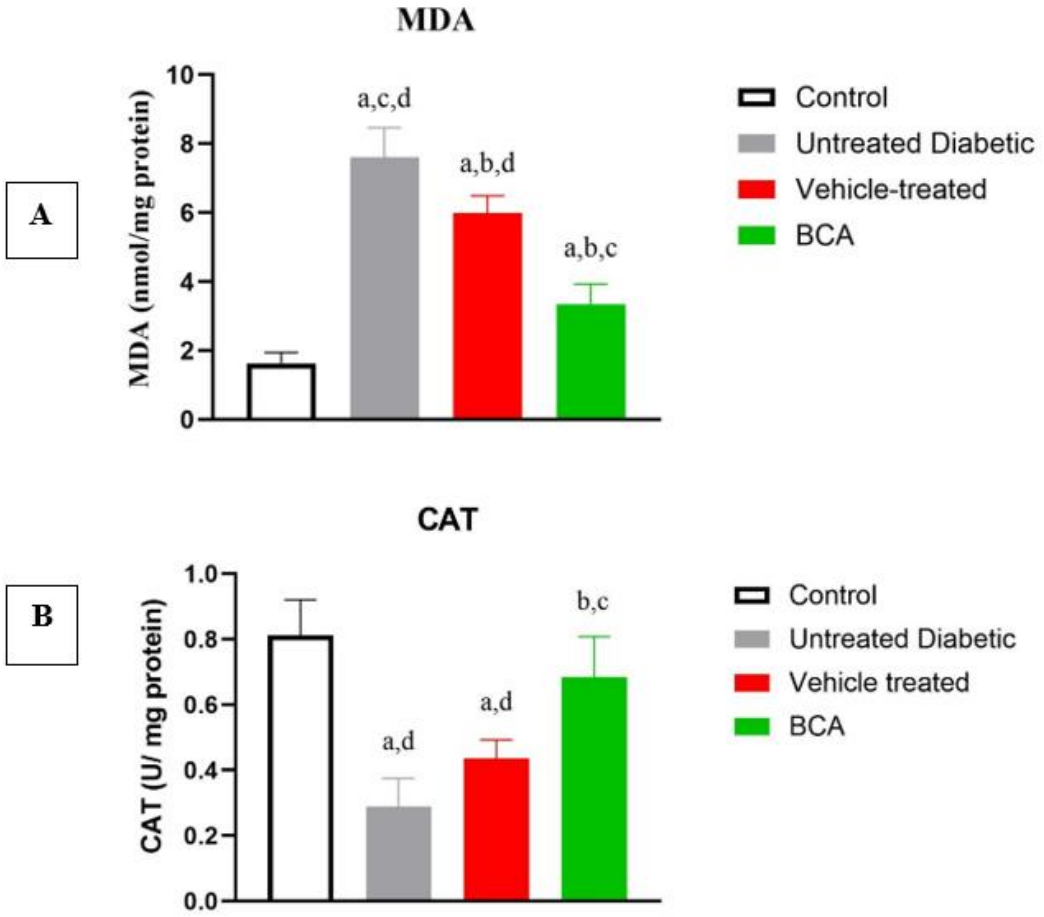

Figure 1: Graphic presentation of effect of vehicle-treated, BCA and marketed formulation on oxidative stress and antioxidant markers MDA (A) and CAT (B) concentrations in rats. Each statistics represents the Mean ± SD. Statistically indicate a: Significantly different from Control at p < .05 b: Significantly different from Untreated Diabetic at p < .05 c: Significantly different from Vehicle-treated at p < .05 d: Significantly different from BCA-treated at p < .05.

BCA increases expression of Nrf2, and NQO1

In the present study, the effect of Biochanin A (BCA) on the Nrf2/ARE pathway was evaluated by measuring the expression levels of Nrf2 and NQO1 in the tissue of different experimental groups via immunohistochemistry. The results demonstrated that the untreated diabetic group exhibited a significant decrease in both Nrf2 and NQO1 expression (Figure 2) compared to the control group.

When diabetic rats were treated with BCA, the treated group showed a remarkable and significant restoration of Nrf2 expression levels, reaching levels comparable to those observed in the control group (Figure 2). Similarly, BCA treatment led to a significant increase in NQO1 expression, reaching levels slightly higher than the control group and significantly higher than both the untreated diabetic and vehicle-treated groups (Figure 2).

In contrast, the vehicle-treated group showed modest improvement in Nrf2 and NQO1 expression levels compared to the untreated diabetic group, yet remained significantly lower than the Biochanin A-treated group.

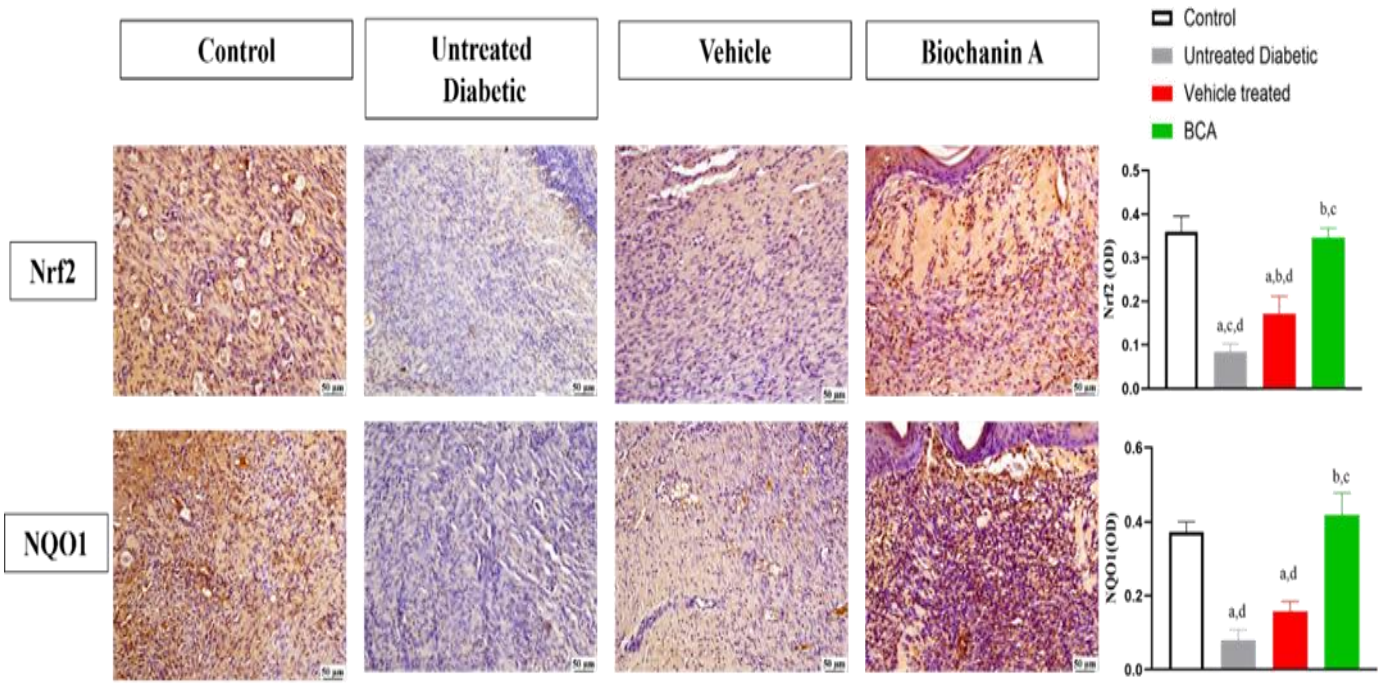

Figure 2: Effects of Biochanin A on the expression levels of Nrf2 and NQO1 in rats tissues. Representative immunohistochemical images showing the expression of Nrf2 and NQO1 in tissues from different experimental groups: Control, Untreated Diabetic, Vehicle-treated, and Biochanin A (BCA)-treated groups. The graphs on the right show quantitative analysis of Nrf2 and NQO1 expression levels measured as optical density (OD). Scale bars = 50 µm. a: Significantly different from Control at p < .05 b: Significantly different from Untreated Diabetic at p < .05 c: Significantly different from Vehicle-treated at p < .05 d: Significantly different from BCA-treated at p < .05.

4. Discussion

Diabetes is a chronic metabolic disorder with numerous etiologies that is characterized by hyperglycemia resulting from insulin deficiency and resistance. In general, the cutaneous manifestations of diabetes appear after the development of the disease, but they may also be the first signs or even precede the manifestations of the primary condition by several years (18).

Patients with diabetes have a compromised antioxidant system due to the decreased expression of ROS-detoxifying enzymes. Oxidative stress, in turn, accelerates the progression of diabetes and its complications (19).

This study demonstrates that Biochanin A (BCA) effectively mitigates oxidative stress within the skin tissue of diabetic rats. A key finding was the significant elevation in the activities of the antioxidant enzyme CAT in the skin of diabetic rats following BCA treatment. These enzymes represent a crucial cellular defense against reactive oxygen species (20). Our observations are consistent with previous research by (21), who reported similar enhancements in CAT activity in streptozotocin-induced diabetic rats treated with BCA, reinforcing the compound's antioxidant potential in diabetic models.

Diabetes-induced hyperglycemia is known to increase free radical formation, leading to heightened oxidative stress and consequent lipid peroxidation (22). Malondialdehyde (MDA), a major secondary product of lipid peroxidation, can cause cellular damage through cross-linking with proteins and DNA and may contribute to the formation of advanced glycation end products (23). In the present study, we observed significantly elevated MDA levels in the skin of diabetic rats, indicative of increased lipid peroxidation. Notably, BCA treatment effectively reduced these elevated MDA levels, demonstrating its capacity to counteract lipid peroxidation in diabetic skin. This finding aligns with the work of (15), which also confirmed BCA's ability to decrease MDA levels and bolster antioxidant defenses.

Furthermore, our investigation delved into the molecular mechanisms underlying BCA's protective effects. We found that BCA treatment led to increased expression of the transcription factor Nrf2 and the phase II detoxifying enzyme NQO1 in diabetic skin tissue. The Nrf2/ARE pathway is a critical regulator of cellular antioxidant responses. Our results corroborate the findings of (15), who showed that BCA exerts protective effects against oxidative stress via activation of the Nrf2 pathway and subsequent upregulation of NQO1. The significance of this pathway in mediating BCA's antioxidant effects is further highlighted by a comprehensive review (17), which documented the consistent antioxidant properties of BCA across various experimental settings, often linked to the modulation of the Nrf2/ARE pathway.

5. Conclusion

In summary, our findings indicate that BCA mitigates oxidative stress in the skin of diabetic rats by enhancing the activity of crucial antioxidant enzyme CAT, reducing lipid peroxidation (MDA levels), and activating the protective Nrf2/NQO1 signaling pathway. These results underscore the potential of BCA as a therapeutic agent for managing oxidative stress-related skin complications associated with diabetes.

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