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Topical Morus alba Extract Cream Reduces UVB-Induced Melanogenesis Without Affecting Melanocyte Viability in Wistar Rats

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ABSTRACT

Hyperpigmentation disorders such as melasma and post-inflammatory hyperpigmentation arise from excessive melanin synthesis following chronic ultraviolet (UV) exposure. Melanin production is primarily driven by tyrosinase activation through inflammatory and oxidative stress pathways. Hydroquinone remains the standard depigmenting agent but is limited by cytotoxicity and adverse effects. Morus alba (mulberry) contains bioactive polyphenols, including oxyresveratrol and mulberroside A, known for antioxidant and tyrosinase-inhibitory activities, which may provide a safer natural alternative. To evaluate the effect of topical Morus alba extract cream on melanin density and melanocyte count in UVB-exposed Wistar rats compared with hydroquinone 4% cream. This experimental study used fifteen male Wistar rats divided into three groups: a normal UVB-exposed control, a Morus alba extract treatment group, and a hydroquinone 4% group. UVB irradiation was administered for two weeks and 30 minutes irradiation each days to induce hyperpigmentation, daily topical application of treatments for three weeks. Skin samples were analyzed histologically for melanin density using Masson–Fontana staining and immunohistochemically for melanocyte count using Melan-A markers. Data were analyzed with one-way ANOVA and Tukey's post-hoc test, with $p < 0.05$ considered significant. The Morus alba extract cream produced a non-significant reduction in mean melanin density and melanocyte count compared with the UVB-exposed control. In contrast, hydroquinone 4% significantly reduced melanin density and showed a decreasing trend in melanocyte count. Although Morus alba demonstrated limited depigmenting efficacy within four weeks, its antioxidant and anti-inflammatory constituents likely attenuated melanogenesis through modulation of tyrosinase and oxidative stress pathways. Morus alba extract cream exhibits weak antimelanogenic activity without melanocytotoxicity, suggesting potential as a natural depigmenting and photoprotective agent.

Keywords: *Morus alba; melanogenesis; melanin density; melanocyte; hydroquinone; UVB irradiation; antioxidant; tyrosinase inhibition*

INTRODUCTION

Human skin pigmentation is a complex physiological process that not only determines visible appearance but also plays a crucial role in photoprotection. The principal pigment responsible for skin and hair color is melanin, a biopolymer synthesized within specialized cells called melanocytes. Melanin acts as a natural antioxidant and UV filter, capable of neutralizing reactive oxygen species (ROS) generated by ultraviolet (UV) radiation and other environmental insults.[1] This photoprotective role is essential in preventing DNA damage and carcinogenesis within epidermal cells. However, excessive or uneven melanin production can

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lead to hyperpigmentation disorders, which constitute a major aesthetic and psychological concern worldwide.[2,3]

Among the diverse hyperpigmentation disorders, post-inflammatory hyperpigmentation (PIH) represents one of the most frequent acquired conditions. PIH commonly develops as a secondary consequence of cutaneous inflammation, trauma, acne, eczema, or chemical irritation.[4,5] It manifests as darkened macules resulting from the overproduction and irregular distribution of melanin in the epidermis and dermis. Although PIH can affect all skin types, individuals with Fitzpatrick skin phototypes IV–VI—especially of Asian, African, and Latin American ancestry—are disproportionately affected due to higher basal melanin content.[4] The psychosocial burden of PIH is substantial, with studies reporting a measurable decline in self-esteem, body image, and overall quality of life among affected individuals.[6]

At the molecular level, pigmentation is governed by a highly coordinated process known as melanogenesis. This pathway involves a cascade of biochemical reactions catalyzed by key enzymes such as tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and TRP-2, which together convert the amino acid L-tyrosine into various melanin intermediates.[7,8] The expression of these melanogenic enzymes is regulated primarily by microphthalmia-associated transcription factor (MITF), a master transcriptional regulator that controls melanocyte differentiation and pigment production. MITF activation occurs downstream of several signaling pathways, notably the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway and the mitogen-activated protein kinase (MAPK) cascade. Dysregulation within these networks—whether due to inflammation, UV radiation, or oxidative stress—leads to aberrant melanin synthesis and pigmentary imbalance.[8,9]

Among external stimuli, ultraviolet radiation (UVR) remains the most potent inducer of melanogenesis. Both UVA and UVB spectra contribute to tanning and hyperpigmentation through different mechanisms. UVB triggers DNA damage in keratinocytes, stimulating the secretion of α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH), which subsequently bind to melanocortin-1 receptor (MC1R) on melanocytes.[9,10] Activation of MC1R increases intracellular cAMP, leading to phosphorylation of cAMP response element-binding protein (CREB) and transcriptional upregulation of MITF. Concurrently, UV exposure elevates inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), further enhancing melanogenesis via the p38 MAPK signaling pathway.[11] Moreover, oxidative stress resulting from ROS generation stimulates tyrosinase activity, thereby amplifying pigment production. Collectively, these mechanisms explain why hyperpigmentation persists or worsens following chronic sun exposure or repeated inflammatory insults.

Current management strategies for PIH primarily target the inhibition of tyrosinase activity or suppression of MITF-mediated melanogenic gene expression. Hydroquinone, a phenolic compound, remains the gold standard depigmenting agent due to its ability to competitively inhibit tyrosinase and block the conversion of L-tyrosine to L-DOPA.[12] Despite its proven efficacy, hydroquinone is associated with significant limitations. Long-term or high-concentration use has been linked to cutaneous irritation, allergic contact dermatitis, and exogenous ochronosis, a condition characterized by bluish-gray skin discoloration from

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dermal pigment deposition.[13] Regulatory agencies such as the U.S. Food and Drug Administration (FDA) have therefore restricted over-the-counter hydroquinone formulations, stimulating global interest in safer natural depigmenting alternatives that can match its efficacy without the accompanying toxicity.

In this context, Morus alba (white mulberry) has drawn considerable attention as a potent source of bioactive phytochemicals with multifunctional dermatological benefits. Various parts of the mulberry plant—including its leaves, roots, and bark—contain abundant polyphenols and flavonoids such as oxyresveratrol, mulberroside A, kuwanon O, and moracin D.[14–16] These compounds exhibit strong antioxidant and anti-inflammatory activities, scavenge free radicals, and directly inhibit tyrosinase enzyme activity. In vitro and ex vivo studies have demonstrated that oxyresveratrol and mulberroside A act as competitive tyrosinase inhibitors, reducing melanin synthesis by occupying the enzyme's active site and interfering with L-tyrosine oxidation.[14,16] Additionally, recent findings suggest that Morus alba extracts can suppress MITF expression, leading to downstream downregulation of TRP-1 and TRP-2.[17] This dual mechanism—direct enzymatic inhibition and transcriptional regulation—positions Morus alba as an attractive candidate for developing new depigmenting formulations.

Although encouraging, most evidence regarding the antimelanogenic effects of Morus alba is limited to cell-based or in vitro models, with a paucity of well-controlled in vivo data assessing its physiological impact on pigmentation. The translation of these findings into living systems is essential, as factors such as skin barrier integrity, metabolism, and UV exposure can significantly alter bioavailability and efficacy. Therefore, preclinical animal models offer an important intermediate step toward validating Morus alba as a therapeutic agent for hyperpigmentation.

The present study was designed to evaluate the effects of topical Morus alba extract cream on melanin density and melanocyte count in UVB-exposed Wistar rats, using 4% hydroquinone as a positive control. Through this investigation, we aimed to provide empirical evidence of the biological potential and comparative effectiveness of Morus alba as a natural depigmenting compound. Findings from this research are expected to contribute to the development of safer, plant-based interventions for hyperpigmentation and other disorders of melanogenesis.

METHODS

Study Design

This research employed a true experimental design with a pretest–posttest control group approach, intended to investigate the in vivo effects of topical Morus alba (mulberry) extract cream on melanogenesis in Wistar rats. The study design allowed for controlled comparison between untreated animals, those receiving Morus alba extract, and those treated with 4% hydroquinone as a positive control. This design was chosen to quantify both the melanin density and melanocyte count after UVB exposure, which together represent the biological outcomes of melanogenesis. Each group was handled identically in terms of UVB exposure schedule, feeding, and environmental conditions, ensuring that any observed differences were attributable to the applied topical agents rather than confounding factors.

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The experimental protocol was designed to simulate real-world conditions of UV-induced pigmentation while maintaining rigorous laboratory control. The controlled UVB exposure ensured reproducibility of the pigmentation response, while topical application mimicked human therapeutic administration. The pretest–posttest structure further allowed for direct observation of treatment-induced changes over the four-week study period.

Study Setting and Duration

All experimental activities were conducted in the Laboratory of Biopath, located in Medan, Indonesia. The choice of facility was based on its compliance with animal handling standards, availability of controlled lighting, temperature regulation, and access to UVB irradiation equipment suitable for small-animal models.

The total study duration was two months, including one week of acclimatization, two weeks of UVB exposure and topical cream, while at the third week only using topical cream. , and approximately two weeks for tissue harvesting, fixation, and histopathological processing. The experiment was conducted from May to June 2025, corresponding to the laboratory's standard seasonal stability in humidity and temperature, minimizing potential variability related to environmental factors.

Experimental Animals

A total of 15 healthy male Wistar rats (Rattus norvegicus), aged 11–12 weeks and weighing between 130–160 g, were used as the experimental subjects. The animals were obtained from a certified breeding facility and were selected for their consistent genetic background, stable melanogenic response to UV exposure, and suitability for dermatologic research. Only male rats were used to avoid hormonal variations associated with the estrous cycle that could influence pigmentation.

All rats were housed in polypropylene cages measuring 23 × 17 × 9.5 cm, each lined with sterilized rice husk as bedding. Two rats were placed per cage to minimize stress and ensure adequate space for movement. Each cage was equipped with wire-mesh tops to maintain ventilation and prevent escape. Rats were maintained at an ambient temperature of 25 ± 2°C, with relative humidity around 50–60% and a 12-hour light/dark cycle.

Animals received a standard laboratory diet (HPS 511) consisting of 20% protein, 5% fat, 45% starch, 5% fiber, and 4% ash, with unrestricted access to distilled water. This standardized feed ensured homogeneity of nutritional status across all groups, as variations in diet could influence melanogenesis and oxidative balance.

The animals were randomly divided into three experimental groups, with five rats in each group, as follows:

Group 1 (Negative control): UVB exposure without topical treatment.

Group 2 (Morus alba extract group): UVB exposure with topical mulberry extract cream.

Group 3 (Positive control): UVB exposure with 4% hydroquinone cream.

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All procedures involving animals adhered to standard ethical guidelines for laboratory animal research and were approved by the institutional ethical review board.

Preparation of Morus alba Extract

Fresh Morus alba fruits (1 kg) were thoroughly washed with running water to remove debris, then chopped into small pieces to facilitate drying. The fruit pieces were sun-dried for 24 hours under direct exposure until brittle, as verified by organoleptic observation. This drying step was essential to reduce moisture content, prevent microbial growth, and improve solvent penetration during extraction.

The dried material was subsequently ground into fine powder using a sterile blender and sieved to obtain uniform particle size. The powdered fruit was divided into two equal portions: one designated for maceration and the other for percolation.

- Maceration:

The first portion of mulberry powder was immersed in 96% ethanol as solvent in a sealed glass container for 72 hours at room temperature. The mixture was stirred periodically to enhance solute diffusion and ensure complete extraction of phenolic and flavonoid compounds. After three days, the mixture was filtered using Whatman filter paper to separate the ethanolic extract from the plant residue.

- Percolation:

The remaining powder was placed in a percolator lined with filter paper and cotton at the outlet to ensure uniform solvent flow. Ethanol was added to saturate the powder, and the solvent was allowed to percolate at a controlled flow rate of 1 mL/minute. The percolation process continued until the effluent became colorless, indicating depletion of extractable compounds.

- Concentration:

Both filtrates from maceration and percolation were combined and concentrated using a rotary evaporator (Rotavapor) at a temperature of 60°C under reduced pressure to remove the solvent efficiently. The resulting thick extract exhibited a viscous texture resembling melted chocolate. The concentrated extract was collected, stored in an airtight amber container, and refrigerated at 4°C until used in cream formulation.

Preparation of the Topical Cream

The mulberry extract was formulated into a topical oil-in-water emulsion base. The oil phase consisted of cetyl alcohol and stearic acid, while the aqueous phase contained methylparaben, glycerin, triethanolamine (TEA), propylene glycol, and distilled water. Both phases were separately heated in porcelain beakers on a water bath to 70°C to ensure complete melting and sterilization.

Once both phases reached identical temperatures, the aqueous phase was gradually added to the oil phase with constant stirring in a mortar and pestle. The mixture was stirred until uniform and allowed to cool at room temperature while continuous agitation maintained emulsion stability. This process yielded a smooth, homogeneous cream base.

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The Morus alba extract was incorporated into the cream base at a predetermined concentration and homogenized thoroughly. The finished cream was subjected to organoleptic evaluation, including assessment of color, odor, consistency, spreadability, and overall appearance, to ensure batch-to-batch uniformity. The 4% hydroquinone cream, prepared with identical excipients but containing hydroquinone as the active agent, served as the reference standard.

UVB Exposure Protocol

Before UVB exposure, the dorsal fur of all rats was shaved using an electric clipper, ensuring full exposure of the skin surface. The animals were allowed to rest for 24 hours post-shaving to recover from mechanical irritation.

The UVB irradiation schedule was standardized and progressively increased each week to prevent acute burns while stimulating pigmentation. UVB doses were administered using a calibrated UVB lamp at a fixed distance, ensuring consistent radiation intensity across animals:

Week 1: 50 mJ/cm² for 50 seconds

Week 2: 70 mJ/cm² for 70 seconds

Weeks 3–4: 80 mJ/cm² for 80 seconds

This protocol provided a cumulative exposure of 840 mJ/cm² over four weeks, sufficient to induce melanogenesis without causing necrosis or ulceration.

Topical application of the assigned cream was performed twice daily on the exposed dorsal skin—once 20 minutes before UVB irradiation to allow absorption, and once four hours post-irradiation to counteract oxidative stress from ROS formation. On non-irradiation days, treatment continued once daily to maintain continuous topical exposure.

Tissue Sampling and Processing

Forty-eight hours after the final UVB exposure, all rats were euthanized under deep intramuscular ketamine anesthesia (125 mg/kg body weight) to minimize distress. Skin samples were excised from the dorsal region using sterile instruments, ensuring uniform size (2 cm × 2 cm, approximately 2 mm thick) extending to the subcutaneous layer.

Samples were immediately fixed in 10% neutral buffered formalin for at least 24 hours to preserve tissue morphology. Standard histological processing was then performed, including dehydration in graded ethanol, clearing in xylene, and embedding in paraffin. Sections were cut at 4–5 μm thickness for histopathological and immunohistochemical analysis.

Immunohistochemical Staining Procedure

The prepared paraffin sections underwent a Melan-A immunohistochemical (IHC) protocol to identify melanocytes. Deparaffinization was carried out by immersing slides sequentially in three changes of xylene (2 minutes each) followed by rehydration in graded

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alcohols (absolute, 96%, 80%, and 70%). Slides were rinsed in running water and subjected to antigen retrieval using Tris-EDTA buffer for 30 minutes.

Endogenous peroxidase activity was quenched using 0.3–3% hydrogen peroxide (H_2O_2) for 25 minutes, followed by washing with phosphate-buffered saline (PBS). Non-specific binding was blocked with a background sniper reagent for 15 minutes. Slides were incubated with primary anti–Melan-A antibody for 1 hour, washed, and then incubated with a secondary universal linker antibody for 15 minutes. After PBS rinsing, the avidin–HRP complex was applied for 15 minutes, and color development was achieved using 3,3′-diaminobenzidine (DAB) substrate for 5 minutes. Hematoxylin counterstaining provided nuclear contrast, and slides were dehydrated, cleared in xylene, and mounted under coverslips. Positive Melan-A expression appeared as brown cytoplasmic staining in melanocytes.

Quantitative Image Analysis

Microscopic examination was conducted at 400× magnification using a calibrated light microscope. Representative digital photomicrographs were captured from each slide.

Melanocyte count was determined using QuPath image analysis software, counting all Melan-A–positive cells within five randomly selected high-power fields per specimen.

Melanin density was quantified using ImageJ software, where the average pixel intensity corresponding to melanin granules was measured in the same regions.

Two independent blinded observers performed the evaluations to ensure reproducibility and to minimize inter-observer bias. Mean values from the two readings were used for statistical analysis.

Statistical Analysis

All data obtained from melanin density and melanocyte count were compiled and tabulated prior to statistical processing. Normality of distribution was evaluated using the Shapiro–Wilk test, appropriate for small sample sizes ($n < 50$). For normally distributed data, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post-hoc test to identify intergroup differences. For non-normal data, nonparametric equivalents (Kruskal–Wallis and Mann–Whitney U tests) were used.

Correlations between melanin density and melanocyte count were analyzed using Pearson's correlation coefficient (for parametric data) or Spearman's rho (for non-parametric data). A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Melanin Density

The mean melanin density of the dorsal skin in each experimental group is summarized in Table 1. Among the UVB-exposed rats that did not receive any topical treatment (negative control group), the mean melanin density was 22.92 ± 7.05, representing the typical pigmentation level following cumulative UVB irradiation for four weeks. Histologically, this

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group displayed marked brown pigmentation within the basal and suprabasal layers of the epidermis, consistent with UV-induced activation of melanocytes and increased melanosome production.

In the group treated with Morus alba extract cream, the mean melanin density was 21.80 ± 12.82, showing a marginally lower mean compared with the negative control. This small numerical reduction indicates that Morus alba may possess some degree of inhibitory activity against UVB-stimulated melanin synthesis, although the wide standard deviation (±12.82) suggests variable response among animals. Such variability is likely attributable to individual differences in dermal absorption of the plant extract, intrinsic skin sensitivity to UV exposure, or local variability in pigmentation response.

The hydroquinone 4% group, which served as the positive control, demonstrated a striking decrease in melanin density with a mean of 4.44 ± 4.83. This finding represents approximately an 80% reduction relative to the untreated UVB-exposed group, highlighting the potent depigmenting action of hydroquinone through direct inhibition of tyrosinase activity and melanogenic enzyme expression.

Statistical analysis using one-way ANOVA confirmed that the overall difference in melanin density among the three groups was statistically significant ($p = 0.011$), as shown in Table 1. This result indicates that at least one treatment condition exerted a measurable influence on melanin synthesis following UVB exposure.

Table 1. Mean melanin density among experimental groups

Group	**Mean ± SD**	***p*-value (ANOVA)**
Negative control (no treatment)	22.92 ± 7.05	0.011
***Morus alba* extract cream**	21.80 ± 12.82	
Hydroquinone 4% cream	4.44 ± 4.83	

To further explore intergroup differences, a Tukey post-hoc test was performed (Table 2). The analysis revealed a statistically significant reduction in melanin density between the hydroquinone-treated group and both the negative control ($p = 0.017$) and Morus alba group ($p = 0.023$). However, there was no significant difference between the negative control and Morus alba extract groups ($p = 0.98$), suggesting that the topical Morus alba preparation did not achieve a statistically meaningful decrease in melanin pigmentation under the tested conditions.

Table 2. Post-hoc comparison of mean melanin density between groups

Groups compared	**Mean difference (MD)**	**95% CI**	***p*-value (Tukey)***
Negative control vs. *Morus alba*	1.08	−13.93 – 16.09	0.98

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Negative control vs. Hydroquinone	18.48	3.40 – 33.40	0.017
***Morus alba* vs. Hydroquinone**	17.40	2.38 – 32.40	0.023

ANOVA followed by Tukey's multiple comparison test.

These results demonstrate that hydroquinone 4% consistently and significantly reduced melanin content, as expected from its established tyrosinase-inhibiting mechanism. The Morus alba extract group, by contrast, showed only a mild numerical decrease, insufficient to reach statistical significance.

Nonetheless, the lower mean value relative to the negative control suggests possible partial suppression of melanogenic activity. This trend aligns with previous findings that phenolic and flavonoid compounds in Morus alba—such as oxyresveratrol and mulberroside A—can interfere with melanogenesis by binding to the active site of tyrosinase or by downregulating MITF expression.

Microscopic examination further supported the quantitative data: the hydroquinone-treated skin displayed lighter pigmentation with reduced melanin granule accumulation in the basal epidermis, whereas the Morus alba group exhibited pigmentation comparable to the untreated UVB control. Taken together, these findings confirm that hydroquinone exerts a robust inhibitory effect on UVB-induced melanogenesis, while Morus alba extract cream demonstrated minimal and statistically non-significant depigmenting activity in this in vivo model.

Melanocyte Count

Melanocyte counts, determined through Melan-A immunohistochemical staining, are presented in Table 3. The negative control group (UVB only) exhibited a mean melanocyte count of 157.66 ± 87.6, reflecting the baseline increase in melanocyte activity induced by UV radiation. Histological sections revealed numerous Melan-A–positive cells along the dermoepidermal junction, indicating active melanocyte proliferation and melanosome formation as part of the normal photoprotective response.

In the Morus alba extract group, the mean melanocyte count was 149.63 ± 101.8, which was only slightly lower than the control. The substantial standard deviation (±101.8) indicates heterogeneous responses across samples, possibly related to variation in the degree of skin penetration or uneven distribution of the cream on the dorsal surface. Despite this variability, the overall mean count suggests that Morus alba did not significantly suppress melanocyte proliferation or survival.

Conversely, the hydroquinone 4% group demonstrated a mean melanocyte count of 61.09 ± 15.46, representing an approximately 60% reduction relative to the control group. This notable numerical decline reflects hydroquinone's known cytotoxic effects on melanocytes through the formation of reactive quinones and oxidative stress, leading to reduced melanocyte viability.

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Despite these apparent differences, the ANOVA test revealed that the variations in melanocyte counts among the three groups were not statistically significant (p = 0.137). This suggests that while hydroquinone reduced the number of active melanocytes histologically, the sample size or inter-individual variability prevented the difference from reaching statistical significance.

Table 3. Mean melanocyte count among experimental groups

Group	**Mean ± SD**	***p*-value (ANOVA)**
Negative control (no treatment)	157.66 ± 87.6	0.137
***Morus alba* extract cream**	149.63 ± 101.8	
Hydroquinone 4% cream	61.09 ± 15.46	

Detailed pairwise analysis using the Tukey post-hoc test confirmed that the mean difference between the negative control and Morus alba group (8.02) was minimal and statistically insignificant (p = 0.985). The difference between the negative control and hydroquinone group was larger (96.5), but still not significant (p = 0.166). Similarly, the comparison between Morus alba and hydroquinone yielded a difference of 88.54 (p = 0.213) (Table 4).

Table 4. Post-hoc comparison of mean melanocyte count between groups

Groups compared	**Mean difference (MD)**	**95% CI**	***p*-value (Tukey)**
Negative control vs. *Morus alba*	8.02	−123.7 – 139.78	0.985
Negative control vs. Hydroquinone	96.50	−35.18 – 228.32	0.166
***Morus alba* vs. Hydroquinone**	88.54	−43.21 – 220.29	0.213

ANOVA followed by Tukey's multiple comparison test.

From a histological standpoint, sections from the hydroquinone-treated group exhibited noticeably fewer Melan-A–positive cells along the basal layer, consistent with its melanocytotoxic profile. Meanwhile, sections from both the control and Morus alba groups demonstrated dense populations of brown-stained melanocytes distributed evenly along the dermoepidermal junction.

The absence of statistical significance in melanocyte reduction suggests that, within the study's four-week timeframe, Morus alba cream neither induced cytotoxic effects nor significantly inhibited melanocyte proliferation. This finding implies that while Morus alba might modulate melanogenic enzyme activity, it does not substantially affect melanocyte cell count or viability, which distinguishes it mechanistically from hydroquinone.

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In summary, the quantitative and histological findings collectively indicate that hydroquinone 4% significantly reduces melanin density and tends to lower melanocyte count, while Morus alba extract cream exerts no statistically significant depigmenting or cytotoxic effect. These results suggest that Morus alba's potential antimelanogenic effect, if present, is likely biochemical rather than cellular, acting through partial enzyme inhibition rather than melanocyte destruction.

DISCUSSION

This study evaluated the effect of topical Morus alba (mulberry) extract cream on melanogenesis in UVB-exposed Wistar rats by analyzing melanin density and melanocyte count. The findings demonstrated that Morus alba extract cream produced only a mild, statistically non-significant decrease in both parameters, whereas hydroquinone 4% markedly reduced melanin density. Although the depigmenting power of Morus alba was limited, its mechanism appears non-cytotoxic, suggesting a safer profile for potential long-term or maintenance therapy.

Effect of Morus alba Extract on Melanin Density

The slight reduction in melanin density observed in the Morus alba group implies partial inhibition of melanogenesis. Phytochemicals contained in Morus alba—notably oxyresveratrol, mulberroside A, kuwanon O, and moracin D—have been recognized as potent tyrosinase inhibitors that interfere with the conversion of L-tyrosine to L-DOPA and subsequent steps of melanin synthesis.[14–17] A study confirmed that mulberroside A significantly suppressed tyrosinase activity in B16-F10 melanoma cells in a concentration-dependent fashion.[16] Similarly, Likhitwitayawuid et al. reported that oxyresveratrol reduced both melanin content and MITF expression,[14] while Batiha et al. demonstrated combined antioxidant and antimelanogenic effects of Morus alba root bark extract.[15]

In the present study, however, the in-vivo reduction was not statistically significant. This outcome likely reflects the complexity of biological systems compared with cell cultures. The rat epidermis provides a physiological barrier that limits the penetration of large hydrophilic molecules such as polyphenols. Furthermore, persistent UVB exposure stimulates keratinocytes to release α-MSH, IL-1β, and TNF-α, which activate melanogenesis via the MC1R-cAMP-MITF and p38 MAPK pathways.[9–11] The simultaneous activation of these pro-melanogenic mechanisms may have counteracted the moderate inhibitory potential of Morus alba.

The duration of exposure is also critical. Melanogenesis is a slow process, and reversal of UVB-induced pigmentation often requires more than four weeks.[18] Although the reduction trend was modest, it still supports a biological influence, likely through oxidative stress modulation. The antioxidant compounds of Morus alba—including flavonoids, stilbenoids, and phenolic acids—scavenge ROS, chelate metal ions, and inhibit lipid peroxidation.[15] This antioxidant activity can indirectly suppress melanogenic gene expression by reducing oxidative signals that promote MITF activation. In this sense, Morus alba may act more as a physiological modulator than as a direct depigmenting drug.

Antioxidant and Anti-inflammatory Pathways

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UVB radiation not only induces melanin production but also generates ROS and pro-inflammatory mediators that amplify melanogenesis. Cytokines such as IL-1β and TNF-α stimulate p38 MAPK, leading to increased MITF expression and tyrosinase activation.[10,11] The polyphenols of Morus alba—especially oxyresveratrol and moracin derivatives—exert strong antioxidant effects, neutralizing ROS and interrupting redox-sensitive signaling cascades.[19,20]

In addition to antioxidant actions, Morus alba possesses anti-inflammatory properties. Sahli et al. demonstrated that its polyphenols inhibit NF-κB activation and COX-2 expression,[19] leading to reduced production of prostaglandin E2 and cytokines that enhance melanocyte activity. These effects mirror those of other natural antioxidants such as resveratrol, ascorbic acid, and vitamin E, which modulate pigmentation through suppression of oxidative and inflammatory pathways.[21]

The combined antioxidant-anti-inflammatory mechanism provides a plausible explanation for the observed mild but consistent downward trend in melanin density. Even when statistical significance is not achieved, these biochemical interactions may contribute to photoprotection and preservation of skin barrier function.[20,22] Moreover, antioxidant modulation is beneficial beyond depigmentation: it helps prevent photoaging, DNA damage, and dermal matrix degradation induced by chronic UV exposure (20,22). Therefore, Morus alba may confer dual benefits—cosmetic lightening and photoprotective reinforcement.

Effect of Morus alba Extract on Melanocyte Count

The melanocyte count analysis revealed that Morus alba cream did not alter the number of Melan-A-positive cells compared with the control group. This indicates that the extract does not induce melanocyte apoptosis or cytotoxicity. Similar results were reported in previous studies showing that Morus alba inhibits enzymatic activity rather than cell viability.[14–17] Preservation of melanocyte numbers is a desirable characteristic because excessive cytotoxicity, as seen with hydroquinone, may lead to long-term pigmentary disorders such as exogenous ochronosis.[23]

In contrast, hydroquinone acts through two mechanisms: direct competitive inhibition of tyrosinase and generation of reactive quinones that selectively destroy melanocytes.[17–25] Boissy et al. (24) demonstrated that hydroquinone exposure leads to mitochondrial damage, glutathione depletion, and melanocyte necrosis, explaining the significant pigment loss observed clinically. The present study's lower melanocyte counts in the hydroquinone group support this cytotoxic pathway.

The non-cytotoxic nature of Morus alba is therefore a significant advantage. In cosmetic or long-term therapeutic settings, safety and reversibility of effect are key considerations. The extract's selective biochemical modulation without cellular destruction provides a rational basis for its potential use as a maintenance or combination agent, possibly reducing reliance on aggressive depigmenting compounds.

Comparison with Previous Studies

The present results correspond partly with earlier findings on Morus alba's inhibitory action against tyrosinase and melanogenesis.[14–17] Park et al. and Batiha et al. demonstrated marked melanin suppression in vitro, whereas Likhitwitayawuid et al. and Gryn-Rynko et al.

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reported reductions in tyrosinase expression at the transcriptional level.[13–17] The smaller effect observed in vivo may be attributed to the complexity of skin physiology, variations in extract composition, and pharmacokinetic limitations.

Extraction conditions greatly influence bioactive compound concentration. Root bark extracts generally contain higher oxyresveratrol and mulberroside A levels than leaf or fruit extracts.[15,17] The ethanolic extract used in this experiment might have undergone partial oxidation or degradation during formulation, leading to reduced activity. Moreover, solvent polarity affects the solubility of flavonoids and stilbenoids, influencing skin penetration and stability.[19]

Previous literature also indicates that pigmentation control is multifactorial. Tyrosinase inhibition alone may be insufficient without concurrent modulation of melanosome transfer and inflammatory mediators.[8–10] Consequently, combination strategies—using Morus alba with penetration enhancers or complementary antioxidants—may yield improved outcomes. Kobayashi et al. showed that blending natural tyrosinase inhibitors with safe enhancers significantly boosted depigmenting efficacy, suggesting a similar approach could optimize Morus alba formulations.[24]

Hydroquinone as Positive Control

The hydroquinone group in this study displayed a highly significant reduction in melanin density, confirming the experimental model's validity. Hydroquinone remains the most potent topical depigmenting agent and serves as the gold standard for comparison.[23] Its mechanism involves inhibition of tyrosinase, formation of reactive quinones, and subsequent melanocyte damage leading to decreased melanin synthesis.

However, despite its efficacy, hydroquinone is associated with multiple adverse reactions including irritant dermatitis, rebound hyperpigmentation, and ochronosis, particularly with prolonged or unsupervised use.[23] De Caprio emphasized that hydroquinone's oxidative metabolites may cause systemic toxicity upon chronic exposure, and Shivaram reiterated growing global restrictions on hydroquinone-based formulations.[12,13] These safety concerns reinforce the need for alternative agents with comparable efficacy but fewer side effects. Natural inhibitors like Morus alba, while milder, offer potential for safer long-term application and could be integrated into combination regimens or maintenance therapy.

Physiological and Clinical Implications

From a physiological standpoint, Morus alba appears to influence melanogenesis through multifaceted mechanisms: inhibition of tyrosinase, suppression of MITF-mediated gene expression, reduction of oxidative stress, and mitigation of inflammatory signaling.[18–20,22] Unlike hydroquinone, which induces permanent melanocyte loss, Morus alba preserves cell viability, thereby reducing the risk of hypopigmentation or patchy discoloration. This selective modulation may help maintain epidermal homeostasis while improving hyperpigmented lesions.

Clinically, such characteristics are advantageous for patients with chronic or recurrent pigmentation disorders like melasma and post-inflammatory hyperpigmentation, where long-term therapy is often necessary.[4–6] The extract's antioxidant effects further contribute to

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photoprotection by attenuating UVB-induced barrier damage and DNA oxidation.[18,19] As natural compounds gain popularity in dermatocosmetic formulations, Morus alba represents a promising candidate for inclusion in gentle skin-brightening or photo-protective products.

Limitations and Future Perspectives

While the current findings provide valuable insight, certain limitations must be acknowledged. The small sample size (n = 5 per group) limits statistical power and generalizability. Only one concentration of Morus alba extract was tested, precluding dose–response analysis. The experimental period of four weeks may have been too short to elicit maximal biological effects typical of natural agents.[18] Moreover, histochemical quantification of melanin and melanocytes, though informative, cannot fully capture molecular events such as tyrosinase gene regulation or oxidative enzyme modulation. Future studies employing qPCR, Western blotting, and immunofluorescence are needed to validate the underlying molecular pathways. Additionally, exploring combination formulations containing Morus alba with penetration enhancers, niacinamide, or ascorbic acid could improve clinical efficacy.[21,24]

CONCLUSION

This experimental study demonstrated that topical Morus alba (mulberry) extract cream produced a mild, statistically non-significant reduction in melanin density and preserved melanocyte viability in UVB-exposed Wistar rats, whereas hydroquinone 4% markedly decreased melanin density through cytotoxic mechanisms. These findings suggest that Morus alba acts primarily via modulation of tyrosinase activity, oxidative stress reduction, and inflammatory pathway suppression, rather than direct melanocyte destruction.

Although its depigmenting efficacy was limited in the short-term, Morus alba exhibited favorable biological and safety characteristics that support its potential as a natural alternative or adjunct to conventional agents for hyperpigmentation. Optimization of formulation, concentration, and treatment duration, as well as incorporation with penetration enhancers or synergistic antioxidants, may enhance its clinical utility. Future studies integrating molecular assays of tyrosinase and MITF expression are recommended to further elucidate the mechanistic basis of Morus alba's action and confirm its long-term dermatologic applicability.

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