

Research Article

Dose-Dependent Histopathological and Structural Impact of Chemical Hair-Straighteners on Scalp Epithelium and Hair-Fibres Using Albino Wistar Rats

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ABSTRACT

Chemical hair straighteners are popular for creating straight, smooth hair but contain strong chemicals such as formaldehyde, sodium hydroxide, and parabens, which disrupt the keratin structure by breaking disulfide bonds, critical for hair strength and shape. These chemical interactions often lead to hair and scalp damage, including irritation, inflammation, thinning, burns, and follicular injury. Repeated use has been linked to higher risks of hormone-related cancers, which raises important public health questions. This study investigated how dose and frequency of chemical hair straightener application affect scalp skin and hair fibre histopathology in albino Wistar rats. The research utilized controlled single and multiple topical exposures to a commercial straightener product over various durations. Hair samples were analyzed for porosity and microstructural changes using wet preparation microscopy and a modified buoyancy test, while scalp tissue sections were examined via haematoxylin and eosin staining. Findings showed that hair damage intensified with repeated chemical exposure, progressing from initial cortical fading and cuticular lifting to severe keratin degeneration and pigment loss. Histological analysis of scalp samples revealed dose-dependent effects, including epidermal thinning, keratinocyte necrosis, follicular atrophy and disruption of collagen matrix, all of which worsened with prolonged treatment. These findings underline the cytotoxic potential of chemical hair straighteners and risks related to systemic absorption of their chemical ingredients. Although the study's limitations include the use of an animal model and evaluation of only one product, the results provide a vital basis for further research in humans, chronic toxicity assessment, and development of safer hair cosmetic practices.

Keywords: Chemical hair straighteners; Chemical toxicity; Hair porosity; Hair shaft damage; Scalp histopathology

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INTRODUCTION

The use of chemical hair straighteners has become increasingly widespread worldwide, driven by the desire for cosmetically enhanced smooth and straight hair textures (Needle *et al.*, 2024). These products typically contain potent chemicals such as formaldehyde, sodium hydroxide, parabens, and other reactive compounds, which irreversibly alter the hair's keratin structure by

disrupting the disulfide bonds essential components that confer strength and shape to hair, thereby achieving long-lasting straightening effects (Paula *et al.*, 2022). However, recent research has raised significant concerns regarding the health risks posed by these chemicals, which extend beyond cosmetic alteration to potential harm to both hair and scalp (Chang *et al.*,

2022). Exposure to these agents has been linked to scalp inflammation, irritation, hair thinning, and in more severe cases, burns and follicular damage (Chang *et al.*, 2022). More alarmingly, epidemiological studies have established connections between frequent use of chemical hair straighteners and elevated risks of hormone-related cancers, including uterine, breast, and ovarian cancers (Irungu *et al.*, 2025).

The scalp's delicate epithelial layer is particularly vulnerable to chemical assault, which may induce irritant or allergic contact dermatitis, burns, and multiple histopathological changes such as hyperkeratosis, acanthosis, and inflammatory cellular infiltration (Hwang *et al.*, 2024). These health implications primarily arise from the ability of hazardous substances to penetrate the scalp, damaging epithelial structures and facilitating systemic absorption of endocrine-disrupting chemicals capable of triggering carcinogenic mechanisms ((He *et al.*, 2022). Mechanically, these chemicals weaken the hair shaft by reducing resistance to breakage, creating irregularities in the cuticle, and altering the hair's water retention properties, cumulatively compromising the fiber's structural integrity (Paula *et al.*, 2022). Importantly, the extent of both histopathological and mechanical damage correlates strongly with the frequency and duration of chemical exposure, underscoring the critical need to understand dose-dependent tissue responses (Saadhvi *et al.*, 2024). Accordingly, this study therefore seeks to explore and quantify the structural impact of chemical hair straighteners on the hair and skin, evaluating the extent of epithelial and follicular damage and injury pattern, relating exposure duration to damage severity, and employing histopathological evaluation through standardized staining protocols to quantify tissue changes.

MATERIALS AND METHODS

Study Design

This randomized control experimental study was conducted at the Faculty of Veterinary Medicine, University of Maiduguri, animal house and the Department of Histopathology at University of Maiduguri Teaching Hospital, both located in Maiduguri, Borno State, Nigeria, employing Albino Wistar rats as an *in vivo* model to investigate the histopathological effects of chemical hair straighteners on skin epithelium and hair fibres. The study was conducted with a controlled, randomized design to correlate the extent of tissue damage with varying exposure durations to chemical hair straighteners. Ethical approval was obtained from the Institutional Animal Ethics Committee prior to initiation.

Animals

The animals included in this study were carefully screened to ensure their suitability. Only apparently healthy adult male rats aged 12 weeks, weighing between 180 and 220 grams, and free from any visible skin abnormalities or systemic illness were selected. Rats included in the study exhibited normal behavior, and absence of any prior exposure to chemicals or medications that could potentially confound study outcomes.

Animals presenting with any signs of dermatological conditions on their skin or other body areas were excluded to prevent bias in histopathological evaluation. Similarly, rats demonstrating systemic signs of illness, including lethargy, abnormal posture or movement, or significant weight loss exceeding 10% during the acclimatization period, were excluded. Parasitic infections or other asymptomatic diseases identified during veterinary screening also constituted grounds for exclusion.

Housing: The rats were housed in standard animal cages under standard laboratory conditions with controlled humidity and temperature (12-hour light/dark cycle, temperature $22\pm 2^{\circ}\text{C}$, humidity $55\pm 5\%$) with *ad libitum* access to food and water (Ulfhake *et al.*, 2022).

Acclimatization: Animals were acclimatized for one week before commencement of the experiment.

Chemicals

Hair Straightener Formulation: A commercially available chemical hair straightener containing active ingredients including formaldehyde ($\leq 2\%$), sodium hydroxide, parabens, and other reactive compounds was used.

Control Solution: Normal saline solution was used as a negative control.

Animal Grouping and Exposure Protocol:

Twenty-eight Wistar rats were randomly assigned into seven groups ($n=4$ per group) as follows:

Twenty-eight Albino Wistar rats were randomly assigned into seven groups of four animals each. Group I served as the vehicle control and received normal saline applied with the same volume and protocol as the treatment groups, including a repeated dose administered one week after the initial application. Groups II, III, and IV each received a single topical dose of the chemical hair straightener with exposure durations of 10, 20, and 30 minutes, respectively. Groups V, VI, and VII were administered repeated doses, consisting of an initial application followed by a second identical application one week later, with exposure times of 10, 20, and 30 minutes, respectively.

All groups received thorough rinsing procedure of the treated area after each application to ensure removal of residual product or saline.

Treatment Area and Application Protocol:

The treatment area on the rat's dorsal scalp was carefully marked using a medium plate template measuring approximately 1 cm². The hair in the marked area was gently brushed to loosen existing hair shafts, preserving the natural hair structure while preparing the site for chemical exposure (Dias, 2015).

A volume of 0.5 ml of the chemical hair straightener, equating to a dose density of approximately 0.5 ml/cm², was uniformly applied over the marked 1 cm² area with a sterile applicator, ensuring consistent and thorough coverage (Bellissima, 2024).

Sample Collection:

Following treatment, the rats were sedated using chloroform and subsequently euthanized 24 hours after the final exposure for tissue collection and histopathological analysis (Aguwa *et al.*, 2020).

Hair Samples

Hair shafts were carefully collected from the treated scalp area by trimming with a surgical blade. Each hair sample from individual animals was divided into two portions. One portion was immersed in distilled water to assess the hair shaft porosity by evaluating water absorption capacity, while the other portion was immersed in normal saline and immediately subjected to wet preparation microscopic examination to assess surface morphology and structural integrity.

Porosity of the hair shaft was assessed using a modified buoyancy test adapted from established hair science protocols (Hill *et al.*, 2014). In this test, hair samples were immersed in distilled water and normal saline and observed for their sinking behaviours over a specified time period. The classification of hair buoyancy was based on how quickly and to what extent the hair strands submerged in the fluid. This sinking index serves as an indirect indicator of chemical damage and structural compromise by reflecting alterations in the hair cuticle's ability to retain water. The grading included four levels: Grade 0 (Low Porosity) where the hair remained floating or only partially submerged with minimal sinking; Grade 1 (Mild Porosity) where the hair slowly sank but largely stayed suspended within the fluid column; Grade 2 (Moderate Porosity) where the hair sank to the bottom within an intermediate time frame; and Grade 3 (High Porosity), characterized by hair rapidly sinking to the bottom, indicating high water absorption and porosity (Hessefort *et al.*, 2008). Each hair sample was tested in triplicate, and the results averaged for accuracy.

Skin Biopsy: Full-thickness skin biopsies approximately 1 cm² in size were excised from the treated scalp region and preserved individually in 10% formal saline for histopathological examination.

Histopathological Analysis

Fixation: Skin biopsy samples were fixed in 10% formal saline.

Processing: Samples were processed using a standardized protocol that included dehydration through descending grades of ethyl alcohol, clearing with xylene, and paraffin wax embedding. Sections of 4 µm thickness were cut using Rotary microtome (Leica RM2125) and stained with haematoxylin and eosin (H&E) for evaluation of general tissue architecture (Feldman & Wolfe, 2014).

Hair Shaft Analysis

Structural evaluation of hair fibres was performed by light microscopy to identify cuticle damage, shaft irregularities, and surface morphology alterations using method described by Adya *et al.* (2011).

RESULTS

Table 1 presents how hair porosity changes with increased exposure time to chemical straighteners. The control hair samples, which were untreated, floated indicating no observable damage. Hair exposed to a 10-minute treatment (Group 1) was graded as Grade 0, showing low porosity. With a 20-minute exposure (Group 2), hair demonstrated Grade 1 or mild porosity, indicating some initial structural compromise. Further exposure of 30 minutes (Group 3) and repeated 10+10-minute treatments (Group 4) showed Grade 2 or moderate porosity, reflecting increased cuticle damage and water absorption. Groups exposed for longer cumulative times, 20+20 minutes (Group 5) and 30+30 minutes (Group 6), exhibited Grade 3 or high porosity, characterized by rapid sinking in buoyancy tests indicating significant damage to the hair structure.

Hair Shaft Microscopic Findings

The longitudinal histological panel of hair shaft samples subjected to varying durations and regimens of chemical hair straightener exposure, captured via light microscopy revealed the following:

Plate 1: Normal. (Untreated Control): The control sample displays a well-organized hair shaft structure, featuring a dense and compact cortex, uniformly distributed melanin granules, and a clearly delineated cuticle. The shaft contour is smooth and cylindrical, and the optical density is evenly distributed, indicating a healthy, unaltered architecture.

Figure 2: Ten Minutes (Single Dose) exhibits early cortical fading with minimal structural disruption. Melanin granules remain largely intact but are slightly reduced in definition. The cuticle remains continuous and undisturbed.

Figure 3: Twenty Minutes (Single Dose) demonstrates moderate cortical thinning, with granular

disorganization and pigment fragmentation. The cuticle begins to show partial lifting or erosion, and the shaft outline appears mildly distorted. Structural weakening becomes apparent.

In **Figure 4:** Thirty Minutes (Single Dose), the cortex shows visible vacuolization and bubble-like disruptions, indicative of keratin degeneration. Cuticular erosion is more extensive, and pigmentation is markedly reduced.

Table 1. Buoyancy and porosity Profile of Hair Samples following Chemical Hair Straightener Exposure

Treatment Group	Buoyancy Grade	Sinking Degree	Porosity Interpretation
Control	Floating	Hair remains floating	No observable damage (Baseline)
Group 1 (10 min)	Grade 0	Minimal sinking	Low Porosity
Group 2 (20 min)	Grade 1	Slow sinking, largely suspended	Mild Porosity
Group 3 (30 min)	Grade 2	Intermediate sinking time to bottom	Moderate Porosity
Group 4 (10+10 min)	Grade 2	Intermediate sinking time to bottom	Moderate Porosity
Group 5 (20+20 min)	Grade 3	Rapid sinking to bottom	High Porosity
Group 6 (30+30 min)	Grade 3	Rapid sinking to bottom	High Porosity

Group 4–6 represent repeated exposures with a one-week interval between initial and second treatments.

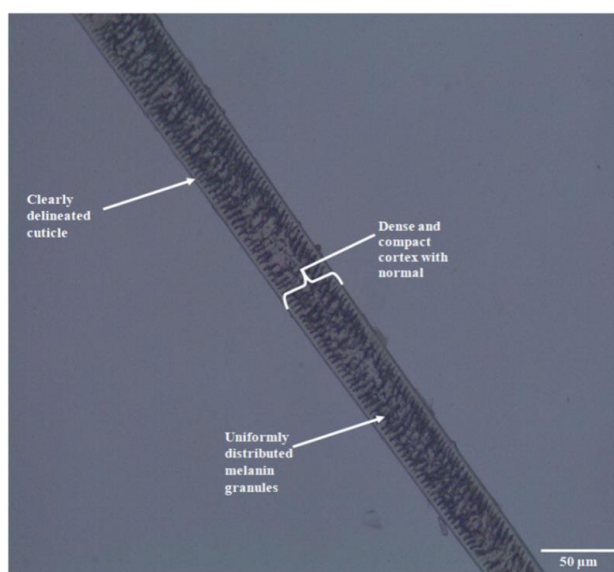


Figure 1: Photomicrograph of Control Hair Shaft Showing Compact Cortex and Intact Cuticle with uniformly distributed melanin granules (Wet Preparation, x100)

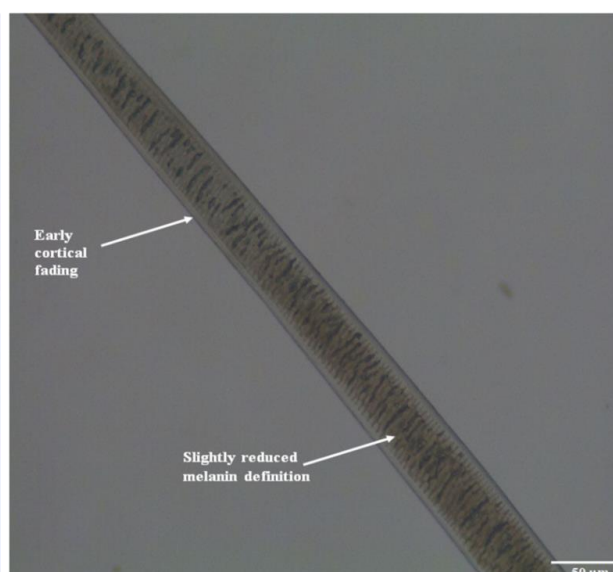


Figure 2: Photomicrograph of Hair after 10-Minute Exposure Demonstrating Cortical Fading and Mild Cuticular Changes (Wet Preparation, x100)

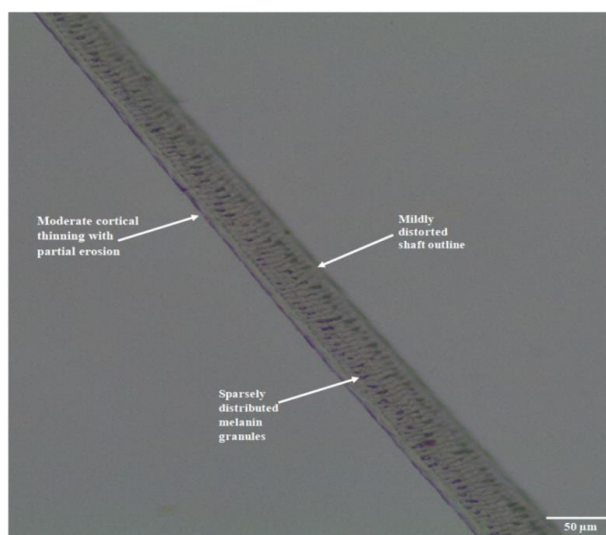


Figure 3: Photomicrograph of Hair after 20-Minute Exposure Showing Cortical Thinning and Partial shaft distortion (Wet Preparation, x100)

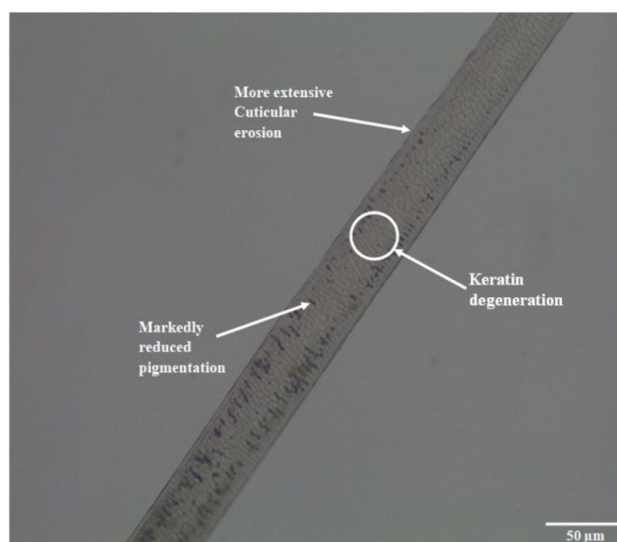


Figure 4: Photomicrograph of Hair after 30-Minute Exposure Illustrating Keratin Degeneration and Pigment Loss with cuticular erosion (Wet Preparation, x100)

Figure 5: Ten plus ten Minutes repeated exposure (1 Week Interval) the hair shows cumulative microdamage, with cortical architecture becoming further disorganized compared to the single 10-minute dose. Pigmentation is patchy and uneven, and cuticular lifting is more pronounced.

In **Figure 6:** Twenty plus twenty Minutes (1 Week Interval), the cortex appears homogenized, lacking

internal detail, with near-complete loss of melanin granules. The cuticle is absent in sections, and the shaft has a rigid, glassy appearance

Figure 7: Thirty plus thirty Minutes (1 Week Interval) displays a completely amorphous cortex with no visible cuticle, and extensive structural distortion. The shaft exhibits signs of bubble hair formation with entirely absent pigment.

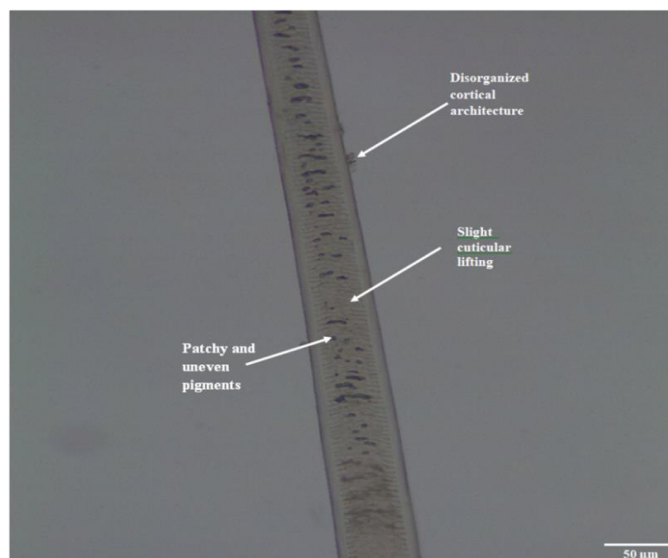


Figure 5: Photomicrograph of Hair after Repeated 10-Minute Exposure Depicting Cumulative Cortical Disorganization and Patchy Pigment Loss (Wet Preparation, x100)

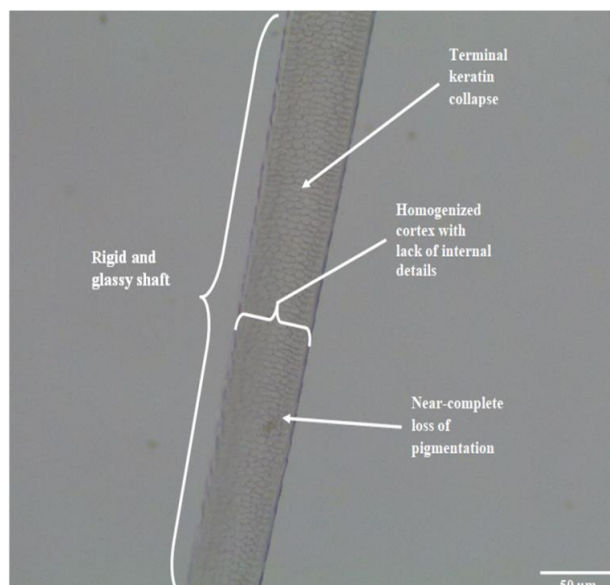


Figure 6: Photomicrograph of Hair after Repeated 20-Minute Exposure Exhibiting Terminal Keratin Collapse and Near-Complete Pigment Loss (Wet Preparation, x100)

Histopathological Findings

Histological evaluation of the skin sections stained with hematoxylin and eosin (H&E) revealed a progressive spectrum of tissue alterations that correlated with the duration and frequency of chemical hair straightener exposure.

Figure 8: Control Group (Untreated Skin) demonstrated the characteristic architecture of normal mammalian skin, consisting of an intact stratified squamous keratinized epithelium overlying a dense collagenous dermis. The epidermis exhibited preserved stratification with a continuous stratum corneum. Dermal appendages including hair follicles were intact

Sections from the 10-minute exposure (Figure 9) demonstrated mild thinning of the suprabasal layers and subtle irregularity of the epidermal-dermal junction. The stratum corneum remained present but slightly loosened. Dermal collagen bundles appeared minimally separated.

By 20 minutes exposure (Figure 10), the epidermis displayed focal degeneration and partial detachment of the upper layers, accompanied by loss of keratinocyte cohesion. The dermis exhibited more pronounced collagen fiber loosening.

The 30-minute exposure (Figure 11) produced more conspicuous damage. The epidermis was markedly thinned with regions of stratum corneum disruption and vacuolization of basal keratinocytes. Dermal changes

included matrix disorganization, early vascular dilation,

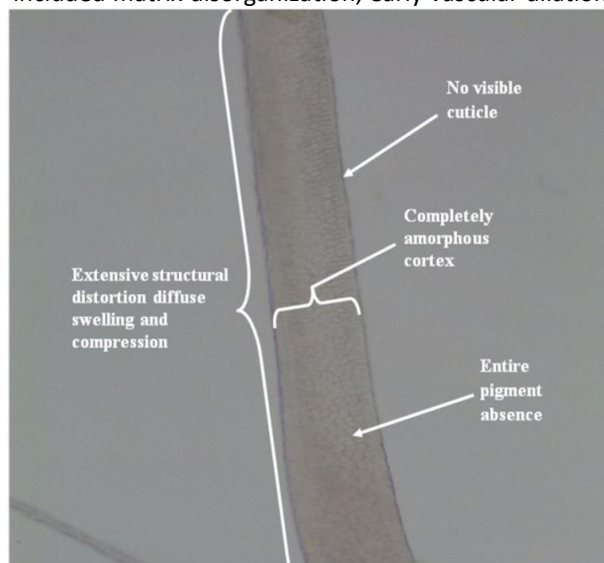


Figure 7: Photomicrograph of Hair after Repeated 30-Minute Exposure Revealing Amorphous Cortex, Absence of Cuticle, and Severe Structural Distortion (Wet Preparation, x100)

and hair follicles appeared distorted.

In **Figure 12:** Ten plus ten Minutes repeated exposure (1 Week Interval), the epidermis appears fragmented and uneven, with partial detachment, the dermis shows a loose and irregular collagen pattern with increased interstitial spaces, hair follicles within the dermis appear distorted, with fewer defined borders compared to typical well-circumscribed follicles

In Figure 13: Twenty plus twenty minutes repeated exposure (1 Week Interval), the epidermis appears notably thinned, exhibited extensive focal ulceration, pronounced ridges and valleys, and hyperkeratotic changes. The dermis showed collagen degeneration, matrix breakdown, marked reduction in visible hair follicles and glandular structures with existing follicles appearing smaller and less defined and lack of defined epidermal and dermal zones.

The most severe lesions were observed in Thirty plus thirty minutes repeated exposure (1 Week Interval) group (Figure 14). The epidermal layer is almost completely absent in regions, with only thin, undulating, fragmented remains at the surface. The dermal region appears loose, with broad acellular spaces and presence of inflammatory cells. Hair follicles and glandular structures are sparsely distributed, small, and irregular, with some visible degradation. Deep fissures or clefts

present, bisecting the tissue and further indicating advanced degeneration

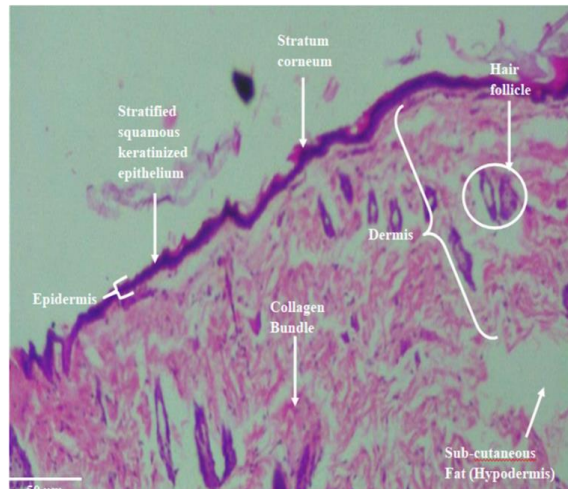


Figure 8: Photomicrograph of control group skin section showing Intact Epidermal and Dermal Architecture (Hematoxylin & Eosin, 100x)

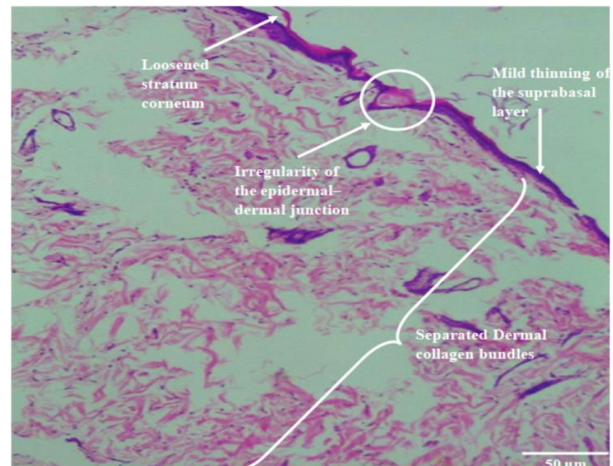


Figure 9: Photomicrograph of skin Section after Single 10-Minute Exposure Showing Early Epidermal Thinning, dermal collagen separation and Irregularity (H&E, 100x)

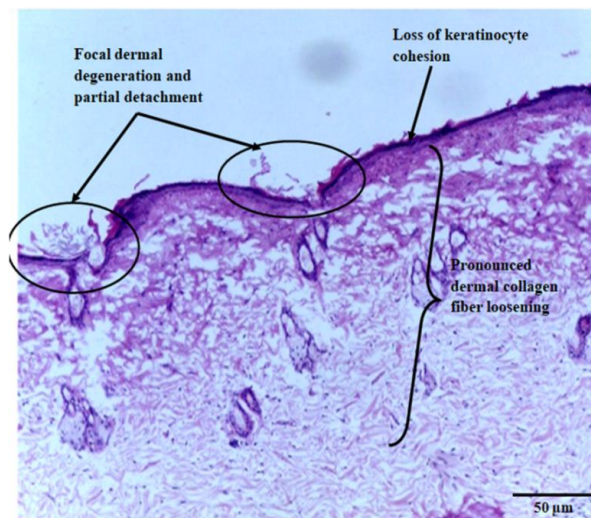


Figure 10: Photomicrograph of skin Section after Single 20-Minute Exposure Demonstrating Showing Focal Epidermal Breakage, Keratinocyte Detachment and Collagen Loosening (H&E, 100x)

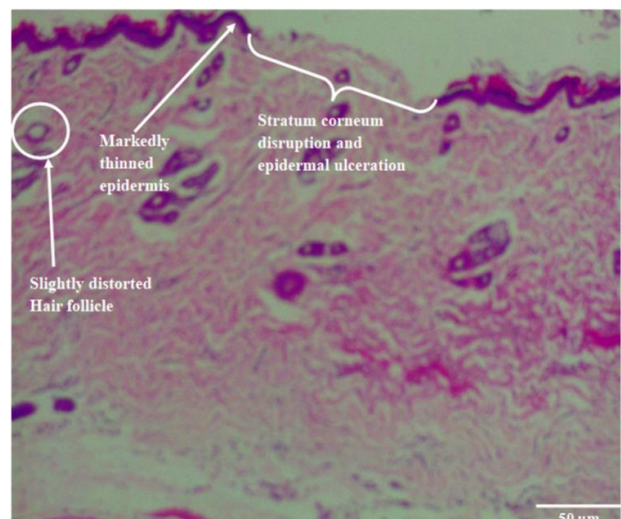


Figure 11: Photomicrograph of Skin section After Single 30-Minute Exposure Showing Epidermal ulceration, Dermal Disorganization and follicular distortion (H&E, 100x)

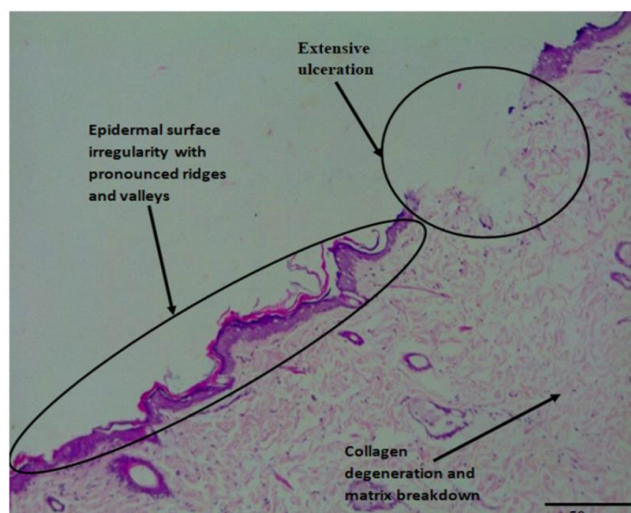


Figure 12: Photomicrograph of Skin Section after Repeated 10-Minute Exposure Demonstrating Epidermal Disruption, Dermal Matrix Alteration, and Follicular Shrinkage (H&E, x100)

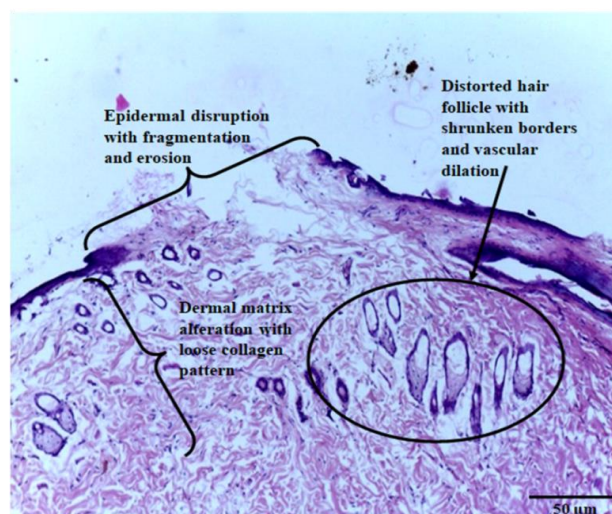


Figure 13: Photomicrograph of Skin Section after Repeated 20-Minute Exposure Showing Severe Epidermal Ulceration, Deep Dermal Damage and Collagen Degeneration, and Loss of Hair Follicles (H&E, x100)

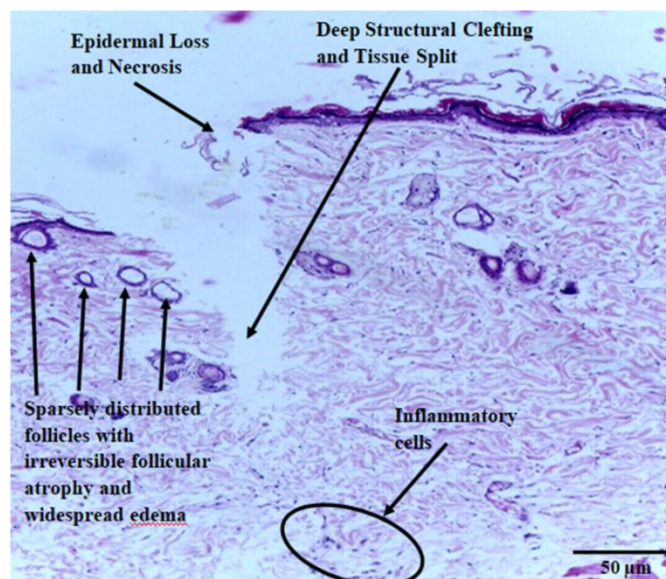


Figure 14. Photomicrograph of Skin Section after Repeated 30-Minute Exposure Showing Severe Necrosis, Dermal Clefting, and Irreversible Follicular Atrophy (H&E, x100)

DISCUSSION

This study highlights the dose- and frequency-dependent histopathological and structural effects of chemical hair straightener exposure on hair fibres and scalp skin in an albino Wistar rat model. Our findings demonstrate progressive hair shaft and tissue degeneration correlating with prolonged and repeated exposures, illustrating the cytotoxic potential of these products.

The observed early cortical fading and cuticular lifting after brief exposure reflect initial keratin disruption, consistent with the chemical alteration of disulfide bonds within hair keratin, a mechanism central to hair strength loss (Cruz *et al.*, 2017).

Progressive structural damage, including vacuolization, pigment loss, and keratin collapse with extended exposure, aligns with increased hair porosity and fragility, supporting previous reports on straighteners

decreasing fiber resilience and augmenting permeability (Gasparin *et al.*, 2025; Seshadri & Bhushan, 2008).

Histopathological changes in scalp skin reveal escalating damage ranging from mild epidermal thinning and collagen loosening at short exposures to severe follicular atrophy, necrosis, and dermo-epidermal clefting following repeated insults. This mirrors chronic irritant dermatitis mechanisms, where continual chemical exposure impedes regenerative processes and drives cumulative tissue degeneration (Patel & Nixon, 2022). The diminished immune infiltration in advanced injury phases possibly indicates immune exhaustion or transition to chronic degenerative states, rather than effective healing, suggesting a compromised cutaneous defense in chronic exposure scenarios (Hatsbach de Paula *et al.*, 2022). Interpreting these findings in the context of human risk requires caution due to interspecies differences: Rat skin differs from human skin in thickness having thinner epidermis, higher follicular density, and different regenerative dynamics compared to human skin, which may influence the susceptibility and damage patterns of chemicals potentially exacerbating exposure effects (O'Brien *et al.*, 2014). Therefore, while these data provide mechanistic insights and hazard identification, direct extrapolation should consider these anatomical and physiological differences.

Regarding the chemical formulation of the straighteners tested, ingredients such as formaldehyde, sodium hydroxide, and various aldehyde derivatives are known for their irritant and cytotoxic profiles. Formaldehyde, for example, can provoke allergic contact dermatitis and has documented mutagenic and carcinogenic potential. (Needle *et al.*, 2024). Sodium hydroxide disrupts protein structures through alkaline hydrolysis, contributing to tissue irritation and cell death. These agent-specific toxicities likely contribute to the observed histopathological changes and hair shaft degradation (Sanad *et al.*, 2019).

While our findings reinforce concerns about chemical hair straightener's local toxic effects, it is essential to clarify that epidemiological associations with cancer risks remain derived from prior population studies rather than direct evidence from this experimental work. Further human clinical trials and chronic exposure studies will be critical to fully define long-term systemic implications.

Together, these results highlight the need for caution and the development of safer cosmetic alternatives through improved formulation and rigorous toxicity evaluations.

CONCLUSION

This study demonstrates the progressive, dose- and frequency-dependent degeneration of hair shafts and scalp skin induced by chemical hair straighteners. The observed cumulative keratin and cuticular damage, increased hair fiber porosity, epidermal necrosis, follicular atrophy, and dermal matrix breakdown contribute valuable mechanistic insights into the localized toxic effects of hydroxide-based straightening products. These findings corroborate previous characterizations of irritant and corrosive damage (Robbins, 2012) and highlight the potential for chronic tissue injury with repeated exposures. While the study did not directly assess tissue recovery over time, the extensive histopathological damage observed highlights the need for minimizing frequent use and developing safer alternatives to protect scalp and hair health.

Limitation

While this study's use of a rat model offers valuable in vivo insights, interspecies anatomical and physiological differences necessitate careful extrapolation to humans. Additionally, only one commercial hair straightener formulation was tested, limiting generalizability, as variability exists among products in chemical composition and concentration. The study's short-term exposure design also precludes assessment of chronic systemic toxicity, carcinogenesis, and reparative molecular pathways, highlighting directions for future research.

Conflict of Interest

The authors declare that there is no conflict of interest associated with the study.

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