International Journal of Scientific Advances
ISSN: 2708-7972
Volume: 6 | Issue: 3 | May – Jun 2025 Available Online: www.ijscia.com
DOI: 10.51542/ijscia.v6i3.30

Topical Testosterone Induces Acne Pilosebaceous Alterations on Rat Skin

Azhar Maulana[1], Willy Sandhika[1,2*]

[1]Immunology Study Program, Postgraduate School, Universitas Airlangga, Surabaya, Indonesia

[2]Anatomic Pathology Department, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia

E-mail: azhar.maulana.nurdhy-2023@pasca.unair.ac.id; willysand@fk.unair.ac.id

*Corresponding author details: Willy Sandhika; willysand@fk.unair.ac.id

ABSTRACT

Background and Aim: Testosterone is a key steroid hormone essential to numerous physiological processes, including male reproductive function and metabolism. While testosterone replacement therapy (TRT) is medically justified in clinically confirmed hypogonadism, an increasing number of over-the-counter (OTC) testosterone products are now marketed with minimal regulation or scientific support. This trend raises concerns regarding potential misuse and unrecognized dermatological risks. Consequently, this study was designed to investigate the safety and potential cutaneous side effects of uncontrolled topical testosterone application, focusing on histopathological skin changes using a rat model. ***Materials and Methods:*** A total of 14 adult male Sprague-Dawley rats, uniform in age and weight, were allocated to a post-test control group design. A defined area on the dorsal skin of each rat was shaved, with the upper half receiving topical testosterone while the lower half served as an untreated control. The animals were then divided into a treatment group (n=7) and a control group (n=7). ***Results:*** Topical testosterone resulted in notable pilosebaceous unit hyperplasia, evidenced by increased sebaceous gland size and hair follicle density. Histopathological evaluation revealed dilated hair follicles filled with keratinous material as a hallmark of comedone formation in acne vulgaris pathogenesis. ***Conclusion:*** These findings demonstrate that topical testosterone induces specific proliferative and keratinization-related changes in the skin. The resemblance to early acne lesions suggests a tangible dermatologic risk associated with Over-The-Counter (OTC) testosterone use, supporting the need for stricter safety assessments and regulatory oversight.

Keywords: human and medicine; drug safety; testosterone.

1. INTRODUCTION

Testosterone, a vital steroid hormone, plays an essential role in various physiological functions, including the development of secondary sexual characteristics and the maintenance of muscle mass. Clinically, testosterone replacement therapy (TRT) is indicated solely for individuals diagnosed with testosterone deficiency (hypogonadism). However, the global market is increasingly saturated with over-the-counter (OTC) testosterone products, often promoted with unsubstantiated scientific claims. This rapidly growing trend, driven by the desire to enhance vitality and performance, raises considerable safety concerns due to potential adverse effects. As the primary androgen, testosterone is recognized for its significant influence on the formation and maintenance of various skin features, including regulation of sebaceous gland activity, hair follicle development, and overall skin texture and thickness. These findings underscore the ability of androgen hormones, such as testosterone, to modulate the visual and functional properties of the skin. Therefore, understanding the visible impacts of testosterone on the outer skin layer is essential to elucidate its broader implications in dermatology. This study aims to investigate and assess the visible changes occurring on the skin surface of rats following testosterone application.

Sebaceous glands, integral components of the pilosebaceous unit, are responsible for producing and secreting sebum a lipid-rich substance that lubricates the skin and hair, contributing to barrier function and hydration [1]. Testosterone is a primary regulator of sebocyte differentiation and sebum production, with increased androgen levels often correlating with enhanced sebaceous gland activity [2]. Furthermore, keratinocytes, the predominant cell type in the epidermis, undergo continuous proliferation and differentiation, forming the protective outer layer of the skin.

Androgens, including testosterone, have been shown to influence keratinocyte proliferation rates and their differentiation pathways, thereby affecting epidermal thickness and texture [3], [4]. Given the well-established role of androgens in normal skin physiology, it is not surprising that these hormones are also critically implicated in the pathogenesis of several common dermatological conditions [4]. Acne vulgaris, a prevalent inflammatory skin disease affecting pilosebaceous follicles, is strongly associated with increased androgen levels, leading to enhanced sebum production, follicular hyperkeratinization, and subsequent inflammation[5]. Similarly, seborrheic dermatitis, a chronic inflammatory condition characterized by scaly, erythematous patches, has also been linked to altered sebum composition and androgenic influence [6]. Among these hormonal regulators, androgens, a class of steroid hormones characterized by their masculinizing effects, play a significant role in the development and maintenance of various skin characteristics [7]. Testosterone, as a principal androgen, is particularly notable for its profound impact on skin biology, influencing sebaceous gland activity, keratinocyte proliferation and differentiation, and the structural integrity of the skin [8],[4]. Studies in various animal models have also demonstrated an association between testosterone levels and observable changes in skin characteristics. For instance, research on male brown bears has shown that seasonal increases in testosterone levels coincide with enlarged sebaceous glands and heightened oily secretions on the dorsal skin [9].

The skin, the largest organ of the body, serves as a vital interface with the external environment, providing protection, regulating temperature, and mediating sensory perception [10], [11]. Its structure and function are intricately regulated by a complex interplay of hormones, growth factors, and cellular signaling pathways [12] including androgens like testosterone. Testosterone significantly influences skin biology, notably impacting sebaceous gland activity and keratinocyte proliferation. This well-established role of androgens in normal skin physiology is further underscored by their critical implication in the pathogenesis of common dermatological conditions such as acne vulgaris, where increased androgen levels correlate with enhanced sebum production and follicular hyperkeratinization. The striking lack of adequate regulation and accurate information regarding the risks associated with non-medical testosterone use necessitates comprehensive research. Given the complex systemic and specific dermatological effects of testosterone, and the potential negative consequences of its uncontrolled use, an in-depth investigation into the safety of OTC topical testosterone is critically important. This study specifically focuses on topical testosterone, increasingly popular in gel or cream formulations, to elucidate its safety profile, toxicity, and potential side effects arising from uncontrolled application. Using a rat model, our investigation aims to assess dose-response relationships, identify skin irritation and allergic reactions, and evaluate precise histopathological and biochemical changes within the pilosebaceous unit, thereby shedding light on its specific dermatological impact.

2. RESEARCH METHOD

This study utilized 14 adult male Sprague-Dawley rats of comparable age and weight. A post-test control group experimental design was employed to investigate the effects of topical testosterone application on rat skin. Following the creation of a shaved square area on the dorsal skin of each rat, the superior portion received topical testosterone, while the inferior portion remained untreated. The rats were randomly assigned to either a control group (n=7) that did not receive testosterone or a treatment group (n=7) that received topical testosterone.

3. RESULTS AND DISCUSSION

Results

Following the creation of a shaved square area on the dorsal skin of each rat, the superior portion was treated with topical testosterone, while the inferior portion remained untreated as a control. On day 0, both areas appeared macroscopically normal, with a uniform skin surface lacking evidence of thickening, discoloration, or lesions. Hair regrowth was evenly distributed across both regions, and no differences were observed between the testosterone-treated and untreated areas. By day 7, the superior dorsal area exposed to topical testosterone demonstrated significant macroscopic changes. The treated skin exhibited marked thickening and erythema, accompanied by noticeably denser and thicker hair regrowth, suggesting androgenic stimulation of the pilosebaceous unit. In contrast, the inferior untreated control area maintained a normal appearance, with an even skin surface, absence of erythema, and uniform hair distribution. These macroscopic differences highlight the specific effects of topical testosterone on cutaneous morphology. Testosterone-treated specimens displayed hypertrophy of sebaceous glands and pronounced hyperplasia of pilosebaceous units, indicative of androgen-mediated stimulation of sebocyte and keratinocyte proliferation. Epidermal analysis revealed acanthosis with increased keratinocyte activity, while the dermis demonstrated thickening and enhanced collagen deposition, likely reflecting fibroblast activation and extracellular matrix remodeling. Hair follicles exhibited dilation and distension with keratinous material, mirroring early comedo formation observed in acne vulgaris. These structural changes suggest that exogenous testosterone induces a hyperproliferative and keratinizing environment conducive to acne pathogenesis. In stark contrast, control specimens without testosterone treatment maintained normal histoarchitecture. The sebaceous glands were small and sparse, hair follicles were limited in number and size, and the dermal connective tissue appeared loose and unremarkable.

Epidermal thickness remained consistent, with no evidence of acanthosis or keratin plugging. This stark dichotomy underscores the specific effects of testosterone on cutaneous structure and function. Collectively, these findings highlight the profound impact of topical testosterone application on the skin, eliciting marked morphological and histological alterations consistent with androgen-driven skin pathology.

The observed features strongly resemble early acne vulgaris, raising potential concerns about the cutaneous safety of topical testosterone, especially in unregulated or over-the-counter formulations. The absence of similar changes in the control areas reinforces the specificity of the androgenic effects, emphasizing the need for cautious evaluation of testosterone's role in skin physiology and pathology.

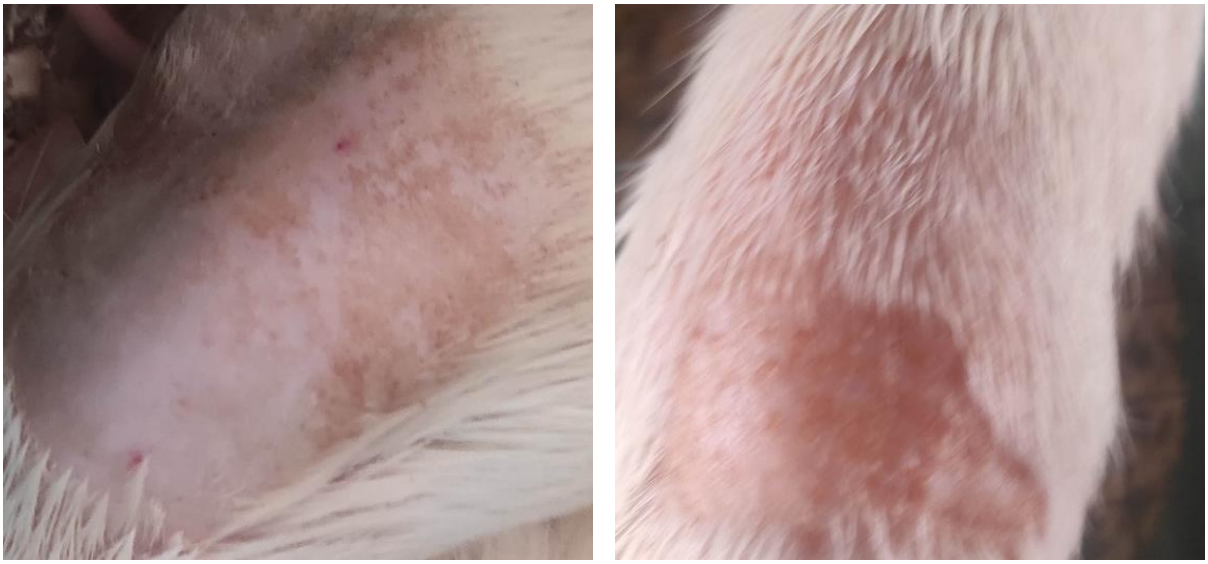

FIGURE 1: Rat's skin before testosterone topical application (left)
Rat's skin after testosterone topical application for 14 days (right).

Microscopic examination revealed distinct and significant alterations in the cutaneous architecture of rats subjected to topical testosterone treatment, compared to untreated controls. In the testosterone-treated specimens, sebaceous gland hypertrophy and pronounced follicular hyperplasia were consistently observed, indicating a robust androgenic stimulation of the pilosebaceous unit. These findings were further supported by a marked increase in the density of sebaceous and follicular structures. Epidermal sections revealed prominent acanthosis, characterized by keratinocyte hyperproliferation, while the dermis exhibited thickening with increased collagen deposition, indicative of fibroblast activation and extracellular matrix remodeling. Moreover, hair follicles in the testosterone-treated areas displayed notable dilation and distension filled with keratinous material, resembling early comedo formation a hallmark of acne vulgaris pathogenesis. Additionally, dermal layers showed augmented fibrocollagenous matrix deposition and increased vascular proliferation, features that underscore the tissue's response to androgenic stimuli. These observations are indicative of early hyperplastic changes driven by androgen exposure. In stark contrast, the untreated control specimens preserved normal cutaneous architecture. The sebaceous glands appeared small and sparse, hair follicles were limited in number and size, and the dermal connective tissue was loosely organized without signs of hyperplasia, keratin plugging, or vascular proliferation. The epidermis remained uniformly thin, lacking evidence of acanthosis or hyperkeratosis. A clear dose-dependent relationship emerged, where higher concentrations of topical testosterone correlated with more pronounced sebaceous gland hyperplasia and follicular dilation. Collectively, these findings demonstrate that topical testosterone induces distinct and quantifiable changes in skin morphology, mimicking the early stages of androgen-driven skin disorders such as acne vulgaris. The absence of these alterations in untreated controls underscores the specific role of androgenic stimulation in mediating cutaneous pathology. These insights are crucial for understanding the pathophysiology of androgen-mediated skin conditions and have important implications for the clinical use of topical testosterone formulations.

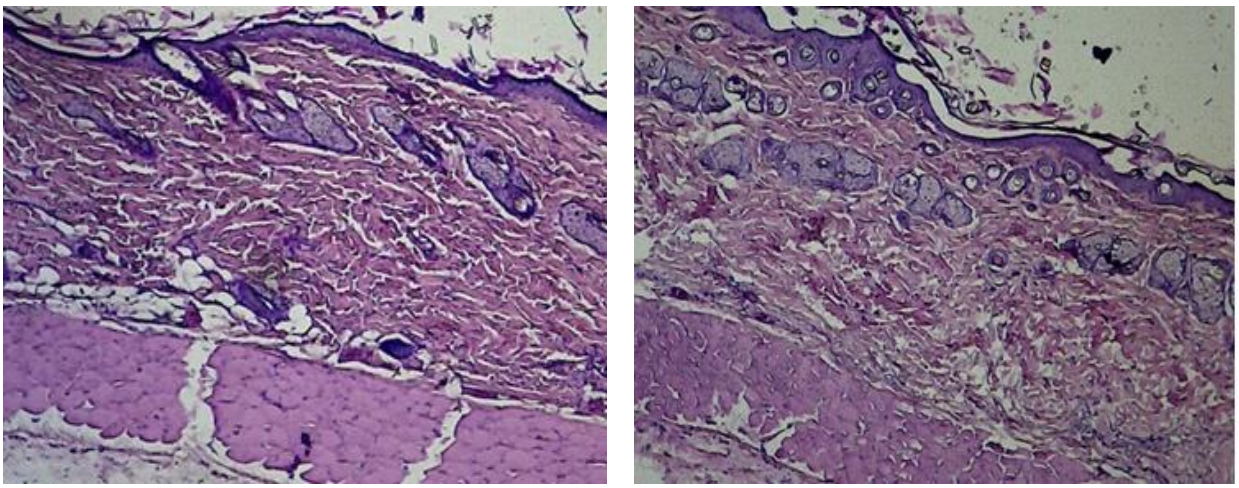

FIGURE 1: Microscopic feature of rat's skin (a) Left: without testosterone topical application on day 14, (b) Right: with testosterone topical application on day 14.

Discussion

Histopathological analysis based on Figure 1 showed significant alterations in the cutaneous architecture of rats following topical testosterone administration, underscoring the profound influence of exogenous androgenic stimulation on skin morphology. Application of topical testosterone consistently induced sebaceous gland hypertrophy and pilosebaceous unit hyperplasia, hallmark features of androgen-mediated cutaneous remodeling [13]. These changes were characterized by the enlargement and increased density of sebaceous glands, accompanied by notable follicular hyperplasia. This morphological response is indicative of enhanced proliferation of sebocytes and keratinocytes under androgenic influence, suggesting activation of androgen receptors in these adnexal structures [14]. Epidermal alterations were also prominent, as evidenced by acanthosis with marked keratinocyte proliferation. These hyperplastic changes suggest a hyperproliferative epidermal response, resembling early-stage androgen-driven dermatoses such as acne vulgaris [15]. In the dermis based on Figure 2 thickening and augmented collagen deposition were observed, likely attributable to fibroblast activation and increased extracellular matrix production in response to testosterone exposure[16]. Vascular proliferation was also noted, further supporting the role of androgens in modulating dermal remodeling and angiogenesis. Hair follicles in testosterone-treated skin based in Figure 2 demonstrated significant dilation and were often filled with keratinous material, a finding reminiscent of comedo formation observed in early acne lesions. This phenomenon underscores the pathophysiological link between exogenous androgen exposure and the development of acneiform changes, providing histological evidence for the role of androgens in initiating follicular obstruction and hyperkeratinization. Importantly, these androgen-induced modifications were absent in the untreated control specimens, which maintained a baseline cutaneous architecture characterized by small sebaceous glands, sparse hair follicles, a thinner dermis with loosely arranged collagen fibers, and a lack of follicular dilation or keratin plugging. The observed histopathological changes suggest a clear dose-dependent response, where higher concentrations of topical testosterone correlated with more pronounced sebaceous gland hyperplasia and follicular dilation. This observation aligns with previous studies demonstrating that androgenic stimulation, particularly via dihydrotestosterone, enhances sebaceous gland activity and keratinocyte proliferation, both of which are central to acne pathogenesis. The findings of this study support the hypothesis that exogenous testosterone induces a localized hyperandrogenic state, thereby promoting sebocyte hyperplasia, keratinocyte hyperproliferation, and follicular plugging histological hallmarks of early acne development [17]. These results also highlight the broader implications of topical androgen use, particularly over-the-counter formulations containing testosterone or its analogs. The morphological changes observed suggest that even localized androgen exposure can induce profound cutaneous remodeling, raising potential concerns about the long-term dermatological safety of such treatments. Furthermore, the activation of androgen receptors in the skin, coupled with increased proliferation of adnexal structures and extracellular matrix components, underscores the need for caution when considering androgen-based therapies for cutaneous conditions[13][18].

4. CONCLUSIONS

This study demonstrates that topical application of testosterone in a rat model leads to significant hyperplasia of the pilosebaceous unit and keratin-filled follicular dilation, findings that closely resemble the early pathological features of acne vulgaris. These results underscore a potential dermatological hazard linked to the unregulated use of testosterone-containing products. In light of these observations, it is imperative to implement rigorous safety evaluations and regulatory controls for over-the-counter testosterone formulations, thereby minimizing the risk of adverse skin reactions associated with their widespread availability.

REFERENCES

[1] Aryani, I. A., Cayadi Sidarta Antonius, Suroso Adi Nugroho, & Nopriyati. (2021). Role of Androgen on Physiological Function of Pilosebaceous Unit. *Bioscientia Medicina : Journal of Biomedicine and Translational Research*, *5*(6), 545–551. https://doi.org/10.32539/bsm.v5i6.321

[2] Szöllősi, A. G., Oláh, A., Bíró, T., & Tóth, B. I. (2017). Recent advances in the endocrinology of the sebaceous gland. *Dermato-Endocrinology*, *9*(1), e1361576. https://doi.org/10.1080/19381980.2017.1361576

[3] Ceruti, J. M., Leirós, G. J., & Balañá, M. E. (2018). Androgens and androgen receptor action in skin and hair follicles. *Molecular and Cellular Endocrinology* 465, (pp.122–133). *Elsevier Ireland Ltd*. https://doi.org/10.1016/j.mce.2017.09.009

[4] Gratton, R., Del Vecchio, C., Zupin, L., & Crovella, S. (2022). Unraveling the Role of Sex Hormones on Keratinocyte Functions in Human Inflammatory Skin Diseases. *International Journal of Molecular Sciences* 23(6). MDPI. https://doi.org/10.3390/ijms23063132

[5] Vasam, M., Korutla, S., & Bohara, R. A. (2023). Acne vulgaris: A review of the pathophysiology, treatment, and recent nanotechnology-based advances. In *Biochemistry and Biophysics Reports* 36. Elsevier B.V. https://doi.org/10.1016/j.bbrep.2023.101578

[6] Dewi, N. P. (2022). Aspek Klinis Dermatitis Seboroik. *Cermin Dunia Kedokteran*, *49*(6), 327–331. https://doi.org/10.55175/cdk.v49i6.241

[7] Alemany, M. (2022). The Roles of Androgens in Humans: Biology, Metabolic Regulation and Health. *International Journal of Molecular Sciences* 23(19). MDPI. https://doi.org/10.3390/ijms231911952

[8] Thiboutot, Diane. "Regulation of human sebaceous glands." *The Journal of investigative dermatology* vol. 123,1 (2004): 1-12. doi:10.1111/j.1523-1747.2004.t01-2-.x

[9] Tomiyasu, Jumpei et al. "Testicular regulation of seasonal change in apocrine glands in the back skin of the brown bear (Ursus arctos)." *The Journal of veterinary medical science* vol. 80,6 (2018): 1034-1040. doi:10.1292/jvms.17-0689

[10] Walker, Mike. "Human skin through the ages." *International journal of pharmaceutics* vol. 622 (2022): 121850. doi:10.1016/j.ijpharm.2022.121850

[11] Liu, K., Wang, Y., Liu, W., Zheng, C., Xu, T., Du, H., Yuan, Z., & Si, C. (2024). Bacterial cellulose/chitosan composite materials for biomedical applications. *Chemical Engineering Journal*, *494*. https://doi.org/10.1016/j.cej.2024.153014

[12] Abdo, J. M., Sopko, N. A., & Milner, S. M. (2020). The applied anatomy of human skin: A model for regeneration. *Wound Medicine* (Vol. 28). Elsevier GmbH. https://doi.org/10.1016/j.wndm.2020.100179

[13] Del Rosso, J. Q., & Kircik, L. (2024). The cutaneous effects of androgens and androgen-mediated sebum production and their pathophysiologic and therapeutic importance in acne vulgaris. *Journal of Dermatological Treatment* (Vol. 35, Issue 1). Taylor and Francis Ltd. https://doi.org/10.1080/09546634.2023.2298878

[14] Zouboulis, C. C., Coenye, T., He, L., Kabashima, K., Kobayashi, T., Niemann, C., Nomura, T., Oláh, A., Picardo, M., Quist, S. R., Sasano, H., Schneider, M. R., Törőcsik, D., & Wong, S. Y. (2022). Sebaceous immunobiology - skin homeostasis, pathophysiology, coordination of innate immunity and inflammatory response and disease associations. *Frontiers in Immunology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2022.1029818

[15] Huang, L., Yang, S., Yu, X., Fang, F., Zhu, L., Wang, L., Zhang, X., Yang, C., Qian, Q., & Zhu, T. (2024). Association of different cell types and inflammation in early acne vulgaris. *Frontiers in Immunology* (Vol. 15). Frontiers Media SA. https://doi.org/10.3389/fimmu.2024.1275269

[16] Nicolas, J., Magli, S., Rabbachin, L., Sampaolesi, S., Nicotra, F., & Russo, L. (2020). 3D Extracellular Matrix Mimics: Fundamental Concepts and Role of Materials Chemistry to Influence Stem Cell Fate. *Biomacromolecules*, *21*(6), 1968–1994. https://doi.org/10.1021/acs.biomac.0c00045

[17] Taieb, A., & Feryel, A. (2024). Deciphering the Role of Androgen in the Dermatologic Manifestations of Polycystic Ovary Syndrome Patients: A State-of-the-Art Review. In *Diagnostics* (Vol. 14, Issue 22). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/diagnostics14222578

[18] Lai, J. J., Chang, P., Lai, K. P., Chen, L., & Chang, C. (2012). The role of androgen and androgen receptor in skin-related disorders. *Archives of Dermatological Research* (Vol. 304, Issue 7, pp. 499–510). https://doi.org/10.1007/s00403-012-1265-x