Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 13 (1)

Histological changes on the skin of guinea pigs administered with *Elaeis guineensis*, Shea butter and hydroquinone base cream (Caro white).

[1]**Oluwayinka P,** [1]**Ujah WO,** [1]**Makanjuola V,** [1]**Ayara C and Duru GO**
[1]Department of Anatomy, Faculty of Basic Medical Sciences,
Bingham University Karu. Nasarawa State

Corresponding Author: Oluwayinka P
Email: oluwadele@yahoo.com; +2347033162631

ABSTRACT
Skin is the largest organ of the body and is known to protect the body from microbes, and helps in thermal regulation. It is divided into epidermis, dermis, and hypodermis. External factors could be due to dermal application of certain chemicals like hydroquinone and exposure to ultraviolet rays. The aim and objective of the study was to identify and detect the occurrence of any physical and histological skin changes caused by application of Elaeis Guineensis, Shea butter and hydroquinone based cream (Caro white) on a skin of guinea pigs. Nine (9) guinea pigs were used and were divided into 7 groups as such; control group, Caro white, Elaeis guineensis, Shea butter and combination of each one with the other for 30 days, 7 days a week applied morning and evening. Macroscopically, erythema, burnt skin and discoloration was seen on the skin of the guinea pigs that was applied with hydroquinone based cream. The red oil and Shea butter showed to have a rapid hair growth effect on the skin of the guinea pigs applied with the mixture. The guinea pigs that were applied with Shea butter showed to have clear skin. Microscopically Koilocytes, hyperplasia of the sweat glands, hyperplasia of the squamous epithelial cells, shrunken keratinized stratified squamous epithelial cells and proliferation of immature hair follicles were seen in the results of the guinea pigs. Conclusion the dermal use of hydroquinone, Elaeis guineensis and their combination has the potential to cause deleterious histological skin changes, while the dermal application of Shea butter has no deleterious effect but has the potential to cause histological skin changes as well.

Keywords: Skin, Elaeis guineensis, Shea butter, Hydroquinone, Guineapigs.

INTRODUCTION
Skin is the largest organ of the body, with a total area of about 20 square feet. The skin protects the body from microbes, it regulates temperature, permit sensations of touch, heat, and cold. The skin has 3 layers, the epidermis, the dermis, and hypodermis. The epidermis, the outermost layer of skin, provides a waterproof barrier and creates a skin tone. The dermis, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands. The deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue[1].

Skin defect associated with cream has been a common thing in the human race most especially with the female gender. Some creams have shown to create an uneven tone on the skin, cause cancer, and other skin defect. Hydroquinone based cream has become a widespread practice which represents the current social phenomenon due to the fact that depigmenting creams, lotions and emulsion are widely available as non-prescription cosmetic preparations in many local market and shops[2]. Creams containing hydroquinone has been said to have caused skin defect such as uneven toning of the skin and making the skin vulnerable due to the fact that it reduces the production of melanin in the body. Studies have shown that hydroquinone can cause exogenous ochronosis, which is an uncommon disorder characterized by a clinical appearance of blue black or gray blue pigmentation but histologically reflects as a yellow brown deposits in the dermis[3]. A lot of side effect has been said to be associated with the use of hydroquinone such as: It causes dryness of the body, It causes irritation to the body, It causes pruritus which is an uncomfortable irritation to the skin that pushes one to scratch, It causes erythema which is redness of the body in a particular area, It causes mild irritant contact dermatitis, constant and lengthy usage of hydroquinone has been noticed to cause ochronosis, a blue-black pigmentation with caviar-like papules on the skin [4].

Dangers of hydroquinone has been discovered to be; Acute Toxicity: There has been at least one case report which suggested that dermal application of hydroquinone might cause peripheral neuropathy [7.] Elaeis guineensis which is widely and commonly known as red palm oil is known to contain Vitamin E, Vitamin K, lauric acid palmitic acid, myristic acid, Carotenoids, phytosterols and stearic. These content most especially vitamin E and palmitic acid have been

known to be an ingredient in many creams and soap. Elaeis Guineensis which is a natural source of Vitamin E (70–80% tocotrienol), is a potent natural antioxidant that can be used in skin-care products. Its antioxidant property protects skin from inflammation and aging [1]. Shea butter a well and commonly used cream in most African homes is extracted from the kernel of the fruit of the shea tree (Vitellaria paradoxa). Shea butter is known to have healing qualities due to the presence of oleic, stearic, palmitic and linolenic acids. It is also known have anti-Inflammatory Properties due to its cinnamic acid content. It is also known to have antioxidant properties: due to its vitamins A and E content. "Shea trees grow wild across 5000km wide belt of savanna[5] There should be discoloration of the guinea pig applied with hydroquinone based cream (caro white), for the guinea pig using red oil, the experiment should be able to clarify its effect and while the guinea pigs using shea butter should show a clear skin due to its natural content

MATERIALS AND METHODS

Twelve (12) healthy adult guinea pigs were kept in well ventilated cages in the Animal House of Bingham University, Department of Anatomy, Karu, Nassarawa State, Nigeria. They were kept in standard laboratory condition; maintained at 35.5-37.0°C ambient temperature and 12:12 hours light and dark cycle respectively. The skin of group II, II, IV, V, VI and VII animal were shaved regularly. The animals were divided into seven groups; some group consist of two guinea pigs while some one:

For Group II, the caro white was topically applied separately on the shaved animals twice daily for 7 days per week and for duration of 30 days. Group III elaeis guineensis and caro white was applied topically on the shaved animals twice daily for 7days per week and for duration of 30 days.

Group IV caro white and shea butter was topically applied on the shaved animals twice daily for 7 days per week and for duration of 30 days. Group V, shea butter was topically applied on the shaved animals twice daily for 7 days per week and for duration of 30 days. Group VI Shea butter and elaeis guineensis was topically applied on the shaved animals twice daily for 7 days per week and for duration of 30 days. Group VII elaeis guineensis only was topically applied on the shaved animals twice daily for 7 days per week and for duration of 30 days.

Animal Sacrifice and Skin Tissue Collection and Processing: Twenty-four hours after the final application, the sites of application were appraised for level of erythema, burnt skin, discoloration, hair growth and other new observations. Photographs were taken for two of the animals of the four groups at the end of every week. Then the animals were sacrificed under anesthesia. The skin of the application site on treated animals and the corresponding site on the control animals were dissected and was fixed in a specimen bottle containing 10% formal saline, after which it was prepared for histological analysis and staining protocols.

Experimental Animal Grouping and Protocol:

Table 1: Experimental Animal Grouping and Experimental Procedure.

Groups	Treatments	Duration (days)	Number
A- Control	Received Water and feed.	30 days	1
B- Caro white only	Received Water and feed and topical application of caro white twice daily	30 days	2
C- Elaeis guineensis and caro white	Received Water, feed and topical application of Elaeis guineensis and caro white twice daily	30 days	2
D- Caro white and Shea butter	Received Water, feed and topical application of Caro white and shea butter twice daily.	30 days	1
E- Shea butter only	Received water, feed and topical application of shea butter only twice daily	30 days	1
F- Elaeis guineensis and Shea butter only.	Received water, feed and topical application of mixture of elaeis guineensis and shea butter only twice daily.	30 days	1
G- Elaeis guineensis only	Received water, feed and topical application of elaeis guineensis only twice daily.	30 days	1

RESULTS

Gross examination: Viewing the skin of the control group through a naked eye examination shows the backs of the guinea pigs having uniform color. The experimental skin shows a weak to moderate patchy hyperpigmentation occurred in most area where the

hydroquinone, mixed hydroquinone and Shea butter and hydroquinone and red oil was applied. The patchy appearance was depicted due to whitish areas of hypopigmentation near other deeply hyper-pigmented and dark spots.

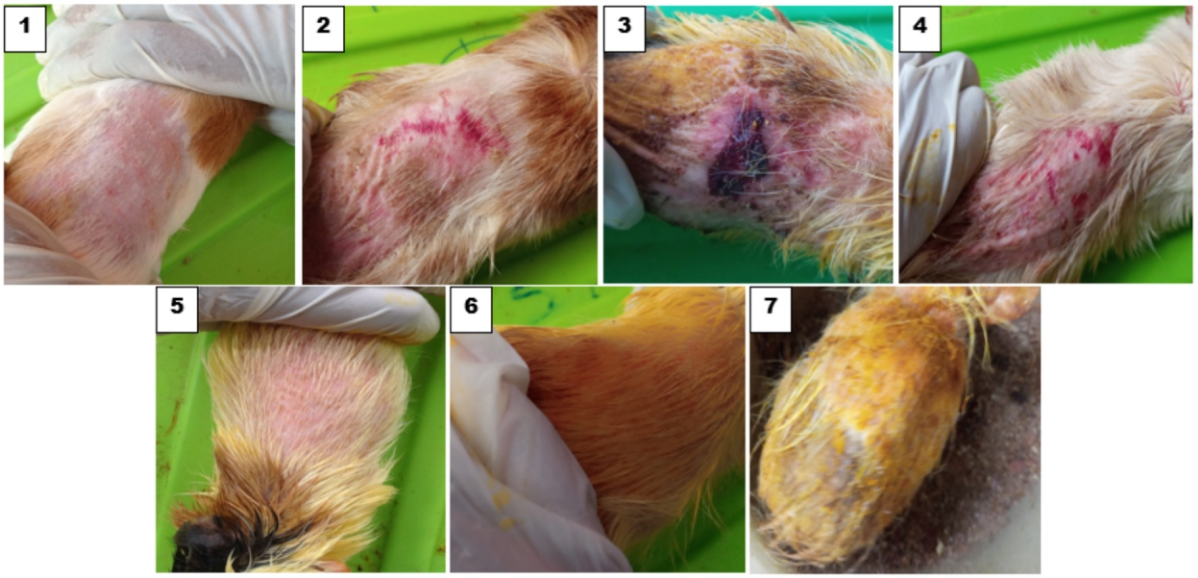

Figure 1: control group (1), showing smooth and clear appearance of shaved dorsum of guinea; 2: hydroquinone base cream group showing skin with gaze of reddish briuse, scaly and thin appearance resulting from dermal irritation after four weeks of application of the cream; 3: hydroquinone base cream and Elaesis guineensis group shows yellow patches on the skin after 4 weeks application; 4: hydroquinone base cream and Shea butter group shows irritation of hair growth around the affected area and red pigmentation of the skin of the affected area after 4 weeks application; 5: Shea butter group showing a clear moderate hair growth after 4 weeks of application; 6: Shea butter and Elaesis guineensis group shows rapid hair growth after 4 weeks application; 7: Elaesis guineensis group showed sparse hair growth after 4 weeks of application

Microscopic Examination Results: Group 1 (control) the skin specimen of the guinea pig of the control group Showed schlerosed and shrunken keratinized stratified squamous epithelial cells with normal sebaceous glands. Hyperplasia of the sweat gland was noticed. Group 2 (Hydroquinone based cream (caro white)) Showed a moderately hyperplastic stratified squamous epithelia cells with mild koilocytosis. The adnexal structures were found to be essentially normal. Mild inflammatory cells were found to be present Group 3 (Elaeis guineesnsis and caro white) Showed a keratinized stratified squamous epithelial cells, with the presence of focal koilocytosis. It showed the proliferation of immature hair follicle. Group 4 (Caro white and Shea butter) Showed stratified squamous epithelial cells with the presence of moderate koilocytosis. The adnexal structures seemed essentially normal. Group 5 (Shea butter only) Showed a moderately hyperplastic stratified squamous epithelia cells with mild koilocytosis. The adnexal structures were found to be essentially normal. Group 6 (Elaeis guineensis and Shea butter) Showed scleroses and shrunken keratinized stratified squamous epithelial cells with normal adnexal structures. Group 7 (Elaeis guineensis only) Showed a keratinized stratified squamous epithelial cells, with the presence of moderate koilocytosis. It showed the proliferation of immature.

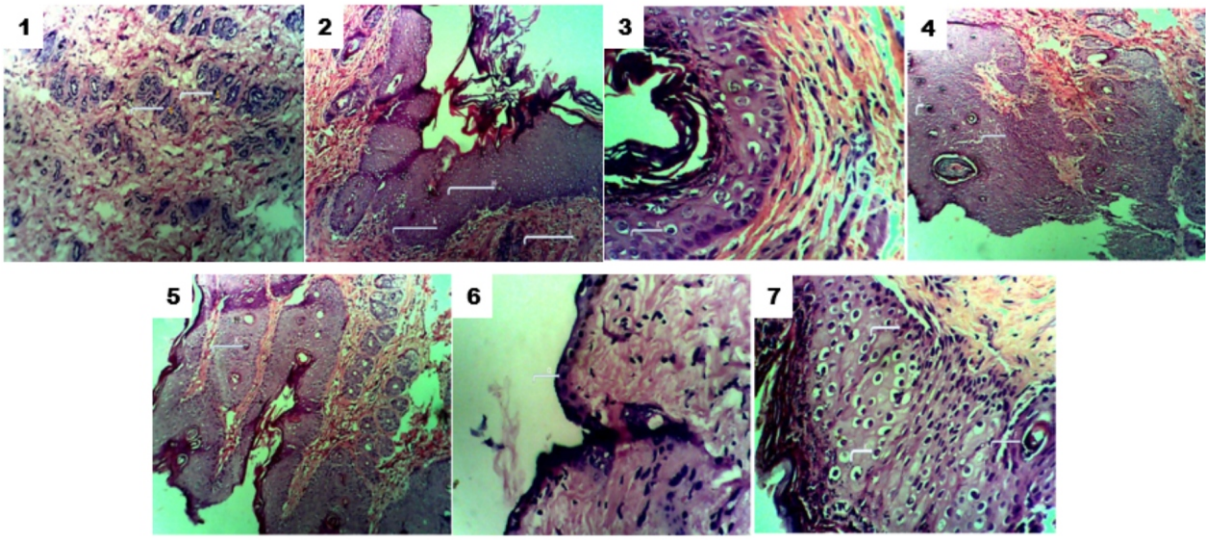

Figure 2: Photomicrographs of the control group skin sections of guinea pigs in group 1 showing normal histology of the skin with sweat gland (S); 2: Photomicrographs of the caro white (hydroquinone based cream) group skin sections of guinea pigs revealing a moderately hyperplastic stratified squamous epithelia cells (H), with mild koilocytosis (K). The adnexal structures were found to be essentially normal. Mild inflammatory cells (I) were found to be present; 3: Photomicrographs of the mixture of caro white (hydroquinone based cream) and Elaeis guineensis group skin sections of guinea pigs showing a keratinized stratified squamous epithelial cells, with the presence of focal koilocytosis (K); 4: Photomicrographs of the mixture of caro white (hydroquinone based cream) and Shea butter group skin sections of guinea pigs showing a (C) stratified squamous epithelial cells (C) with the presence of moderate koilocytosis (K). The adnexal structures seemed essentially normal; 5: Photomicrographs of Shea butter group skin sections of guinea pigs showing a moderately hyperplastic stratified squamous epithelia cells (H) with mild koilocytosis (K). The adnexal structures were found to be essentially normal; 6: Photomicrographs of the mixture of Shea butter and Elaeis guineensis group skin sections of Guinea pigs showing sclerosis and shrunken keratinized stratified squamous epithelial cells (Q) with normal adnexal structures; 7: Photomicrographs of Elaeis guineensis group skin sections of Guinea pigs showing a keratinized stratified squamous epithelial cells (C), with the presence of moderate koilocytosis (K). It showed the proliferation of immature hair follicle (F). (H&E, Magnification X40).

DISCUSSION

The skin of the control group (I) were observed to have retained normal smooth appearance and texture. In the first week, Group II Skin showed a graze injured appearance resulting from dermal irritation caused by the hydroquinone cream, with time the graze appearance was noticed to have reduced and in about the fourth week it was more of a bruised or scar like appearance on the skin. The scaly appearance in the Group II seemed to be noticed from week two and was consistent till the fourth week. In Group III there was an injured/burnt like appearance on the skin, which in the second week seemed to have cleared. On the third week there was an appearance of clot of blood in an affected area and in the fourth week the clot seemed to be more pronounced. There was a presence of yellow patches on the skin due to the Elaeis guineensis presence in the mixture. In group IV the skin seemed to appear burnt in the first week, in the second week it appear to be smoother but had the presence of red patches known as erythema (the reddening of a particular are caused by an irritant) which originated from the burns in the previous week, there was also a Lack of hair growth or inhibition of hair growth around the affected area. The skin appeared to be thinner. In Group V which was shea butter, the skin appeared to be normal, no burns, no injury, there was a normal hair growth noticed throughout the weeks. In group VI the skin appeared normal, with no injuries nor burns. In week 2 and 3 rapid hair growth was noticed. In group VII there was loss of hair as weeks went by, presence of black pigmentation were noticed.

Histological Observation of the Specimens: In group 1 which is the control group was found to have sclerosed and shrunken keratinized stratified squamous epithelial cells. Sclerosis meaning abnormal hardening of body tissue. Meaning the keratinized stratified squamous epithelial cells appeared abnormally hard and shrunken (this could be due to dehydration, or environmental condition such the heat during the summer period and also could be due to bacteria either from the feed or water given to them). Sebaceous glands appeared normal but the Hyperplasia of the sweat gland was noticed in group 1.

There are two types of human sweat glands, apocrine and eccrine. Eccrine glands is found around most part of the body and open directly onto the surface of the

skin. Apocrine glands open into the hair follicle, leading to the surface of the skin. Apocrine glands is found in areas with abundant of hair follicles, such as scalp, armpits and groin. Hyperplasia is the increase in the number of cells. Hyperplasia of the sweat glands in this case is hyperplasia of the eccrine gland. This hyperplasia of sweat glands could be due to the change in feed. In the first week, salad was given as feed which caused excess urine thereby bring about change of their beddings every day. But after two weeks the feed was changed to normal chicken feed, this period there was less urination thereby bring about twice a week change of beddings. Less urination could have caused increase in release of sweat (hyperhidrosis) thereby causing increase in sweat glands [6].

Group II which Showed a moderately hyperplastic stratified squamous epithelia cells is an alteration in epidermal growth characterized by an increase in the number of cells within an epidermis [4]. There are different forms of epidermal hyperplasia which are regular, irregular and pseudocarcinomatous. In regular epidermal hyperplasia the rete pigs are approximately evenly sized and shaped Irregular epidermal hyperplasia the rete pegs are less uniform. Pseudocarcinomatous hyperplasia is a chronic and late stage of epidermal hyperplasia that develops after milder forms (regular or irregular). It develops subsequent to injury. Mild Koilocytosis were found in the skin. Koilocytes are vacuolated keratinocytes which are squamous epithelial cells with perinuclear cavitation and nuclear features of low grade squamous intraepithelial lesions (LSIL) to include nuclear enlargement, coarse chromatin, and irregular nuclear membranes[10]. The adnexal structures were found to be essentially normal. Mild inflammatory cells were found to be present, this inflammatory cells are very important in response to injury in all tissues [8].

Group III and group VII showed similar result. They both have keratinized stratified squamous epithelial cells with the presence of koilocytes (koilocytosis is the structural changes of the epithelial cells) The combination of red palm oil and Caro white showed to have it present and also red palm oil alone showed it present but in group III the koilocytes was focal therefore it shows the caro white increased its effect while group VII showed moderate koilocytes thereby the red palm oil could be a reason for the changes in cell structure) and proliferation of immature hair follicle (immature hair follicle could be caused by the Caro white involvement in the skin thereby destroying the normal functioning of the hair growth and it could also be due to the environment affecting the normal functioning of the skin). They both showed a keratinized stratified squamous epithelial cells, with the presence of (group III) focal koilocytosis and (group VII) moderate koilocytes. It showed the proliferation of immature hair follicle. The Hair Follicle is a vital component of mammalian skin [9]. "HF is involved in sensory perception as a functionally distinct mechanosensory organ, giving the wide tactile sensation range of covered skin surface" [10].

Disorder in hair growth can be attributed, at large, to the changes in the normal dynamic behavior of the HF [11]. The cycle is regulated by various hormones and growth factors produced both inside and outside the follicles, therefore even small environmental changes may lead to a shortening of the anagen, catagen phase induction, and also increasing the number of telogen follicles [12].

Group IV and V showed a similar result, stratified squamous epithelial cells was present in both. But in group V it is moderately hyperplastic with the presence of moderate koilocytosis. In group VI the adnexal structures seemed essentially normal. Showed scleroses and shrunken keratinized stratified squamous epithelial cells with normal adnexal structures.

CONCLUSION

Application of hydroquinone based cream, elaeis guineensis and their mixture shows it has potential to cause deleterious histological skin changes, its appendages and skin damage contrary to the idea that the application of hydroquinone based cream is safe and elaeis guineensis continuous dermal application is safe. In addition, the obtained results shows that pure oils such as Shea butter has no deleterious effect, but has potential to cause histological skin changes as well.

REFERENCES

1. Draelos, E. and Zoe, D. Skin lightening preparations and the hydroquinone controversy. Dermatol. Ther. 2007; 20(5): 308-313.
2. Yoshimura, K., Harii, K., Aoyama, T and Iga T. Experience with a strong bleaching treatment for skin hyperpigmentation in orientals. Plast. Reconstr. Surg. 2000; 105 (3): 1097-1108.
3. Longo, N. Inherited disorders of amino acid metabolism presenting in adults. In: Braunwald, E., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L., Jameson, J.L. (Eds.). Harrison's principles of internal Medicine. 15th edition. New York: Mc Graw Hill. 2001; 2307.
4. Bancroft, J.D. and Stevens, A. Theory and practice of histological techniques. 4th edition. Philadelphia: Churchill Livingstone. 2001; 113-138, 243-267.
5. Maranz, S. and Weisman, Z. Evidence of indigenous selection and distribution of shea trees (vitellaria paradoxa) and its potential significance to prevailing parkland savanna tree patterns in sub Saharan Africa north of equator. J. Biogeogr. 2003; 30:1505-1516.
6. Solish, N., Wang, R. and Murray, C.A. Evaluating the patient presenting with hyperhidrosis. Thorac. Surg. Clin. 2008; 18(2): 133-140.
7. Kooyers, T.J and Westerhof, W. Toxicological aspects and health risks associated with hydroquinone in skin bleaching formula. Ned

Tijdschr Geneeskd. 2004; 148(16): 768-771.
8. King, T.C. Cell injury, Cellular Responses to Injury, and Cell Death. Elsevier's Integrated Pathology. 2007;
9. Brajac, I., Vičić, M., Periša, D. and Kaštelan, M. Human Hair Follicle: An Update on Biology and Perspectives in Hair Growth Disorders Treatment. Hair Transplant. 2014; 4: 115. doi:10.4172/2167-0951.1000115
10. Li, L., Rutlin, M., Abraira, V.E., Cassidy, C. and Kus, L. The functional organization of cutaneous low-threshold mechanosensory neurons. Cell. 2011; 147: 1615-1627.
11. Stenn, K.S. and Paus, R. What controls hair follicle cycling? Exp. Dermatol. 1999; 8: 229-233.
12. Al-Nuaimi, Y., Baier G., Watson, R.E., Chuong, C.M. and Paus, R. The cycling hair follicle as an ideal systems biology research model. Exp. Dermatol. 2010; 19: 707-713.