

## EFFECTS OF COMMONLY AVAILABLE WHITENING CREAMS ON THE HAIR FOLLICLES OF GUINEA PIG SKIN

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### ABSTRACT

**Background:** The trend of lightening the skin is becoming a serious problem. Depigmentation creams are widely available as nonprescription cosmetic preparations in many local markets and shops.

**Objectives:** To determine the effects of over-the-counter available skin whitening creams on the hair follicles of guinea pig skin. To highlight the significance of awareness in general population about the use of non-prescription skin lightening creams.

**Methods:** Sample size of 48 adult, guinea pigs were used in this experiment. The animals were randomly divided into four groups. Group I (control group) this group was applied with no cream and group II, III and IV (experimental groups) all were applied with skin whitening creams A, B and C respectively once every day, six days a week for four- and eight-weeks duration. The skin specimens were examined at histological level.

**Results:** Microscopic examination after 4 weeks duration showed variable degree of increased size of hair follicles from 104.8µm in group I to the maximum of 130.5µm in group III. The effects were exaggerated after eight weeks duration.

**Conclusion:** The use of skin lightening creams caused varying degrees of side effects so damage the skin.

**Keywords:** Skin whitening creams, effects on hair follicles, guinea pig skin.

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### INTRODUCTION

Looking back at the history, humans have been tagged and categorized on the basis of skin complexion<sup>1-2</sup>. In Asian population fair skin is tagged as a symbol of beauty, grace and high social class whereas dark skin is seen as low social status<sup>3</sup>.

Skin lightening creams are used by 59% women in Togo and 27% women in Senegal<sup>4</sup>. According to another study conducted in Malaysia in 2015, a total of 60.6% of female students use skin lightening products<sup>5</sup>.

All the whitening products affect the structure of the human skin due to local application and by exerting harmful effects they hamper skin functions. The diversity in structure of skin is because of environmental influences on skin. The skin comprises of epidermis and dermis. The epidermis is stratified squamous keratinized epithelium having cells called keratinocytes<sup>6</sup>.

The dermis is a connective tissue beneath epidermis. It is further divided into two layers; a superficial papillary dermis and a deep reticular dermis. Papillary dermis is the region present around dermal papillae. It is about 20% of the dermis. It contains loose connective tissue with many capillaries and special receptors for pain, touch and temperature. Reticular dermis is dense irregular connective tissue having collagen and elastic fibers. These are responsible for strength and extensibility of skin. It also contains hair

follicles, sweat and sebaceous glands. Hairs are deficient in thick skin present in palms and soles<sup>6-8</sup>.

These whitening creams mostly contain hydroquinone, steroids and mercury<sup>9</sup>. All these chemicals act as whitening agents by inhibiting the melanogenic enzymes i.e., tyrosinase and related melanogenic enzymes<sup>10-13</sup>.

In countries like Pakistan use of such creams is common due to easy availability of whitening products and a social pressure of fair complexion.

So, an experiment was conducted to see the effects of over-the-counter available whitening creams on guinea pig skin and to emphasize the significance of population awareness in the use of non-prescription skin lightening creams.

## METHODS

Study was of six months duration and it was conducted in the department of Anatomy, King Edward Medical University, Lahore. Sample size of 48 Guinea Pigs was taken. Adult guinea pigs (average age of 2 to 3 months) of weight of 500-1000gms and having different skin color were chosen. They were kept in the animal house and acclimatized for 15 days. A twelve-hour light and dark cycle were maintained at room temperature between 22-25°C. The food (grass hay and chickpeas) and water were provided to these animals ad-libitum. Most commonly used three whitening creams were taken for the experiment and were labelled as A, B and C.

All the animals of sample size were randomly divided into four groups, one control group and three experimental groups. In each group there were twelve animals. All animal groups were further divided into subgroups as I a, I b, II a, II b, III a, III b, IV a, IV b having 6 animals in each subgroup. All animals were marked with different colors for identification.

At the start of experiment a part of skin measuring 3×2 cm on the dorsum of all the animals was cleaned and shaved. Small amount of cream (0.25gm) was taken for each animal and gently spread on the shaved part of skin in all experimental groups.

After four weeks duration 6 animals from subgroup "a" of all groups were anesthetized with chloroform. An elliptical skin area from the treated part was dissected and a specimen of skin over the subcutaneous plane was taken. The margins of skin were stitched with the silk suture. The skin specimens obtained were kept in 10% formalin solution in properly labelled plastic jars.

The animals of subgroup "b" were applied with the same creams in the same pattern for four more weeks. At the end of eight weeks duration similar procedure was done for collection and preservation of samples

for histological examination.

All steps of tissue processing i.e., fixation, dehydration, clearing and wax infiltration and then staining with Hematoxylin & Eosin was done<sup>14</sup>. Findings were noted under light microscopy. Photographs were taken. Study parameters were carefully observed and recorded by micrometry.

The diameter of hair follicles was measured in vertical, transverse and oblique dimensions. Average of these three dimensions was calculated to get the size of hair follicles. Then average of sizes of all hair follicles measured, was calculated. This final value was taken as size of hair follicles in that particular slide.

The data was entered and analyzed by using SPSS version 21.0. Quantitative variable, size of hair follicles was described by using mean ±S.D. Comparison of this quantitative variable was performed by applying One Way ANOVA. P-value ≤ 0.05 was considered statistically significant.

## RESULTS

**Group I:** Control group animals were shaved from their dorsum but no cream was applied on them in whole experimental duration. Observations were noted at four weeks and eight weeks duration. The normal size of the hair follicles measuring  $104.8 \pm 5.5 \mu\text{m}$  as mean size was observed (Table 1, Figure 1).

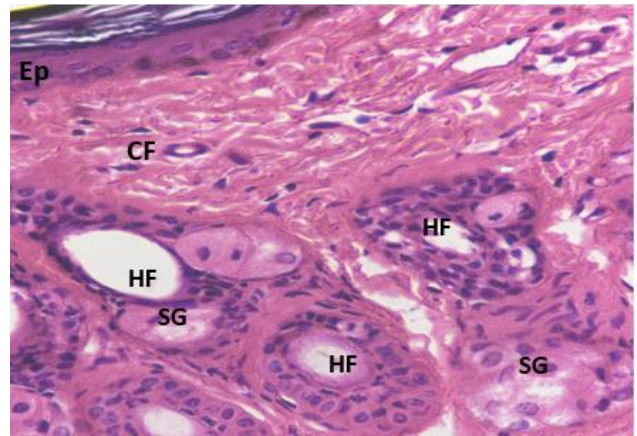


Figure 1: Photomicrograph of control group animal. Epidermis is of normal thickness showing nuclei in 3-4 layers of keratinocytes. Papillary dermis with fine network of collagen fibers (CF). In reticular dermis hair follicle (HF) and sebaceous glands (SG) are visible. (H&E). At magnification of 400X.

### Group II:

**IIa:** Animals of this group were applied with cream A and observed after four weeks of cream application. Hair follicles in this group were enlarged up to

116.9±5.3µms showing active phase of hair growth cycle when compared with the control group.

**IIb:** Animals of this group were applied with cream A and observed after eight weeks duration. At the end treated area of skin showed that hair follicles of this group were markedly increased in size with the mean value of 131.6±15.9µm (Table 1, Figure 2).

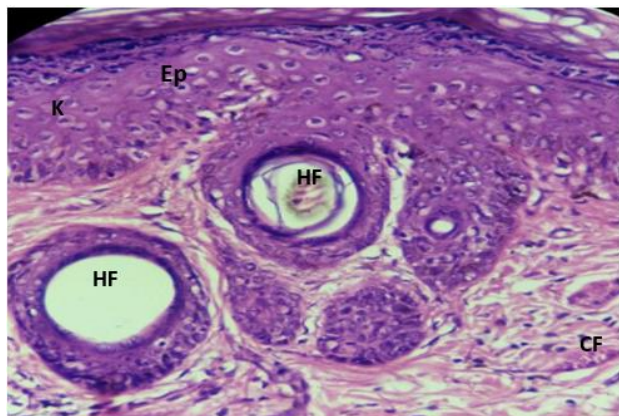


Figure 2: Photomicrograph of group II, thickened epidermis (Ep) with many layers of keratinocytes (K). Papillary dermis showing network of collagen fibers (CF). Large hair follicles (HF) are present in reticular dermis. (H&E). At magnification of 400X.

### Group III

**IIIa:** Animals of this group were applied with cream B and observed after four weeks. At the end of this experiment, treated area of skin was examined. The mean size of the hair follicles was 130.5±8.1µm which was also markedly increased as compared to all other groups (Table 1).

**IIIb:** Animals of this group were applied with cream B and were observed after eight weeks of cream application. Size of hair follicles in this subgroup was largest of all the experimental groups with the mean value of 137.6±8.5µm (Table 1, Figure 3).

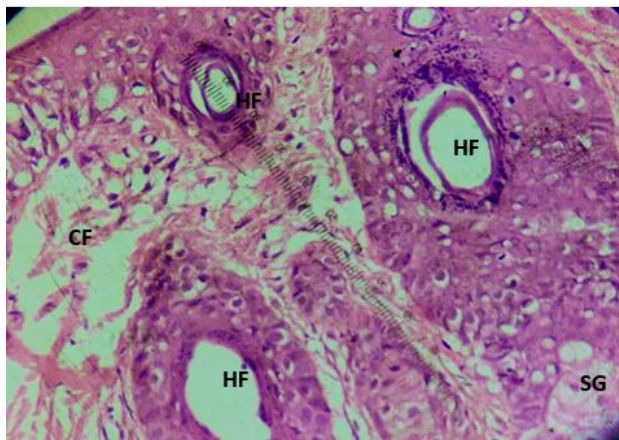


Figure 3: Photomicrograph of group III showing measurement of enlarged hair follicle (HF) and sebaceous glands (SG). Dermal papillae are also visible. Collagen fibers (CF) are in normal distribution in papillary dermis (H&E). At magnification of 400X.

### Group IV

**IV a:** Animals of this group were applied with cream C and were observed after four weeks duration. Mean size of the hair follicle was 110.8±10.4µm (Table 1). This increased size shows active phase of the hair follicles.

**IV b:** Animals of this group were applied with cream C and were observed after eight weeks duration. At the end, experimental area was observed. Mean size of the hair follicle was 115.2±11.4µm (Table 1, Figure 4).

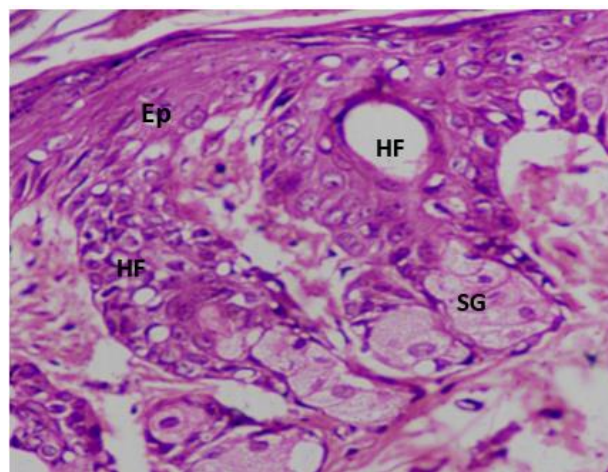


Figure 4: Photomicrograph of group IV having thickened epidermis (Ep). Markedly enlarged multiple sebaceous glands (SG) are seen with hair follicles (HF). (H&E). At magnification of 400X.

## RESULTS

**Size of hair follicles:** After 4 weeks duration the size of the hair follicles was maximum in group III with a mean size of 130.5±8.1µm whereas mean size of hair follicles in group II was observed as 116.9±5.3µm and in group IV was 110.8±10.4µm. After 8 weeks duration the mean sizes of hair follicles were increased in control as well as in experimental groups. Difference of mean sizes between 4 and 8 weeks was significant for group III with p-value 0.035 and insignificant for group I, II and IV with p-values 0.401, 0.060 and 0.337 respectively (Table 1, Figure 5).

When comparison was made among groups, it was found significant with p-values <0.001 and 0.005 at 4- and 8-weeks' time respectively (Table 2).

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Table 1: Comparison between groups for size of hair follicles.

Groups	Size of hair follicles at 4th week (µm)		Size of hair follicles at 8th week (µm)		P-Value
	Mean	Standard Deviation	Mean	Standard Deviation	
Group I	104.8	5.5	110.4	15.8	0.401
Group II	116.9	5.3	131.6	15.9	0.060
Group III	130.5	8.1	137.6	8.5	0.035
Group IV	110.8	10.4	115.2	11.4	0.337

Key: Group I; Control group. Group II; applied with cream A. Group III; applied with cream B, Group IV; applied with cream C

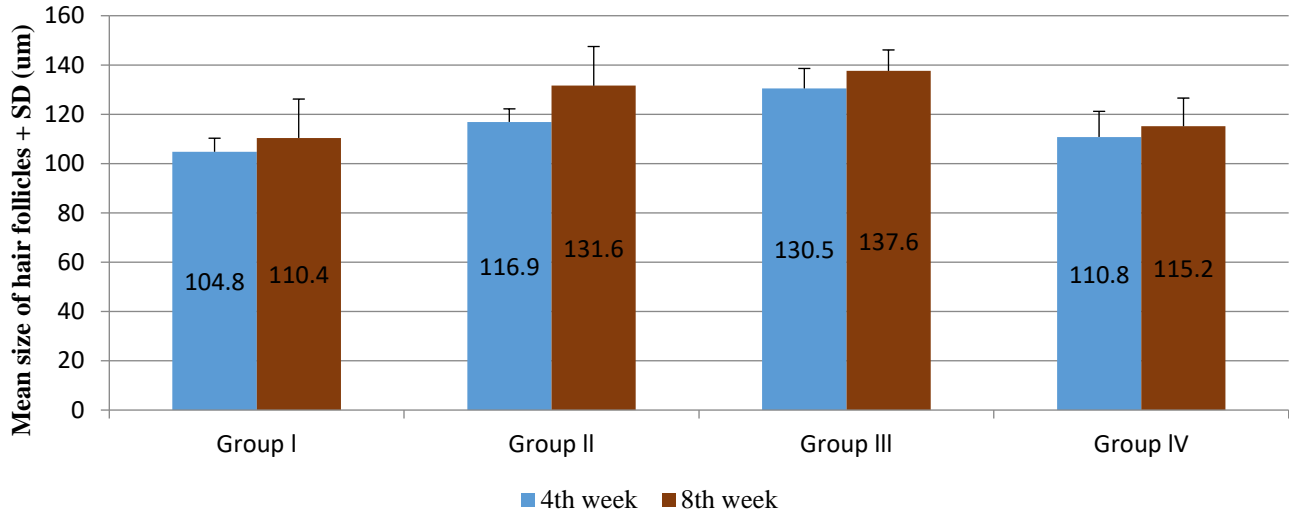


Figure 5: Multiple bar charts presenting mean size of hair follicles along with SD as error bar of four groups at 4 and 8 weeks.

Key: Group I; Control group. Group II; applied with cream A. Group III; applied with cream B, Group IV: applied with cream C

Table 2: Comparison for size of hair follicles among four groups at 4 and 8 weeks (One Way ANOVA).

		Sum of squares	DF	Mean square	F	p-value
4 <sup>th</sup> Week	Between Groups	2187.61	3	729.20	12.47	0.001
	Within Groups	1169.21	20	58.46		
	Total	3356.82	23			
8 <sup>th</sup> Week	Between Groups	3028.56	3	1009.52	5.73	0.005
	Within Groups	3526.08	20	176.30		
	Total	6554.64	23			

Key: DF; degrees of freedom. F test; Ratio of variances

**DISCUSSION**

Multiple skin whitening creams with different names are available in the market. These are mostly without information brochure or disclosure of ingredients. So people using such creams are unaware of their harmful effects.

In the present study the effects of application of three such skin whitening creams on guinea pig skin were observed after application for 4-8 weeks duration.

Ingredients of skin whitening products are usually a combination of hydroquinone, corticosteroids and mercury<sup>15</sup>.

Hypertrichosis was the prominent results of present study. Hair of different areas of the body are in different phases of hair growth cycle. Normal body

hair are in resting phase called telegenic phase. Whitening agents e.g. hydroquinone and steroids cause these hair follicles to enter into active or anagen phase so there is increase in the size of hair follicles in both dimensions that is longitudinal and transverse diameters. The number of hair follicles remains the same in an individual. It's only the phase of hair follicles that can be changed either active or inactive<sup>15-17</sup>. In present study whitening agents are seen to cause activation of hair follicles in all experimental groups at 4- and 8-weeks duration and it is maximum in group III animals.

The results of our study are similar in this respect to previous studies by Vivek Kumar Dey et al in 2014 observed facial hypertrichosis in 18.4%<sup>18</sup>. Another

study by M.A Al-Dhalami et al in 2002 observed hypertrichosis in 19.2% patients<sup>19</sup>.

The results of our study are also very similar to findings reported by Farghaly et al in 2006 in which fifteen guinea pigs were used to see the effects of three whitening creams for 8 weeks duration. They observed increased proliferation of hair follicles<sup>20</sup>.

Although apparently the skin may remain unharmed rather better but actually in deeper layers the changes induced by application of these chemical agents are damaging and harmful.

### ETHICAL APPROVAL

The study was approved by the Institutional Review Board of King Edward Medical University, Lahore via Ref No. 408/RC/KEMU Dated: November 23, 2015.

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### AUTHOR'S CONTRIBUTIONS

**SS, NH:** Conception, Design, Manuscript Writing, Literature Review, Data collection, Data analysis  
Critical review, Proof reading