

## Histological And Histochemical Study Of The Skin Of Female Guinea Pig Under The Influence Of Retinoid

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**Abstract: Introduction:** Vitamin A is important for epithelial cell proliferation and differentiation. Synthetic vitamin A derivatives, known as isotretinoin, have been extensively used in the last few years to treat a variety of clinical skin conditions. **Aim of the Work:** The aim of this study was to evaluate the histological changes in guinea pig thin skin that might result from long term exposure to isotretinoin and the possibility of recovery after its withdrawal. **Material and Methods:** 25 female guinea pig (*Cavia sp.*) were used in this study, 15 of them were adult animals (about 6 months old) and the other 10 were old animals (2- 4 years old). They were divided into five equal groups; 1- control group, 2- adult isotretinoin-treated group, 3- the third group was topically treated with isotretinoin for four weeks, then the animals were left without treatment for another four weeks. 4- Old control group, 5- Old isotretinoin-treated group. Skin specimens from the back were processed and examined histologically and histochemically. The epidermal thickness was measured and the results were statistically analysed. **Results:** Specimens from isotretinoin-treated animals showed antiproliferative effect on the cells having highly proliferative potentiality. The sebaceous glands showed significant decrease in their size. There was also a statistically highly significant decrease in the epidermal thickness. The dermis showed decreased collagen content. It was found that all the effects of isotretinoin are time dependent and all the histological and histochemical manifestations of isotretinoin are reversible. **Conclusion:** Isotretinoin has a time dependent and reversible potent antiproliferative and enzymatic activating effects. Long-term exposure to systemic isotretinoin may induce structural changes in the guinea pig skin, being partially reversible after withdrawal of the drug. Therefore, the use of tretinoin should be restricted to the advice of dermatologists.

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**Key Words:** Isoretinoin, Thin skin, Sebaceous glands.

### 1. Introduction

The retinoids are a class of chemical compounds that are related chemically to vitamin A. Retinoids are used in medicine, primarily due to the way they regulate epithelial cell growth. Retinoids have many important and diverse functions throughout the body including roles in vision, regulation of cell proliferation and differentiation, growth of bone tissue, immune function, and activation of tumor suppress genes (Serri, and Iorizzo, 2008).

There is extensive literature on the use of tretinoin, which is considered to be one of the most potent compounds for treating the signs of aging and/or photodamaged skin, including fine lines, hyperpigmented spots, and wrinkles (Chaudhari *et al.*, 2011).

Retinoids exert profound pleiotropic effects in skin, affecting many aspects of cell differentiation and proliferation. For this reason, retinoids have prominent pharmacological effects on major skin cells (keratinocytes, dermal fibroblasts, melanocytes,

sebocytes) and have shown great potential as therapeutic agents in dermatology. Dermal fibroblasts are important target cells of retinoids and are stimulated to produce extracellular matrix proteins, particularly when skin is damaged by wounding, ultraviolet radiation or glucocorticoids. Retinoids regulate pigmentation and can lighten hyperpigmented skin in animals and humans. Studies with cultured melanocytic cells show that tyrosinase activity is reduced by retinoids. The powerful sebosuppressive effect of some retinoids, such as 13 *cis*-retinoic acid, demonstrates that sebocyte differentiation is altered by retinoids. Retinoids inhibit proliferation and lipid synthesis in cultured human sebocytes and alter their keratin expression (Gendimenico and Mezick, 1993).

Aging of the skin is a complex biological process which is influenced by the interaction of several intrinsic and extrinsic factors. Intrinsic or chronological aging is an inevitable, genetically programmed process, of unclear underlying mechanism, for which no prevention or effective

treatment is currently available. Photoaging refers to the gross and microscopic cutaneous changes that are induced by cumulative exposure to UV radiation and are superimposed on the background of chronological aging (Stratigos, and Katsambas, 2009). Although primarily an aesthetic problem with significant psychological effects, photoaging constitutes the background for the development of precancerous and cancerous skin lesions. Overwhelming clinical and histological evidence indicate that certain structural changes induced by excessive sun exposure can be reversed, to some extent, by the use of topical retinoids. A number of retinoid compounds, for example tretinoin, isotretinoin, retinaldehyde and tazarotene, have been employed for the treatment of photoaged skin, and demonstrate beneficial clinical and histological effects. Adverse effects have been limited to an irritant reaction of variable intensity presenting with dryness, scaling and erythema. (Stratigos, and Katsambas, 2009).

The changes that occur in normal skin after the use of oral retinoid have been overlooked or neglected. Numerous investigations were done regarding the histological affection of the skin after topical use of retinoid (Griffiths *et al.*, 1993) and the effects of various retinoid on various types of skin cells *in vitro* (Vora and Karasek, 1994). However, reports on the effect of systemic retinoid on the skin *in vivo* are few.

The guinea pig (*Cavia sp.*) is a herbivorous rodent which is a relative of rats and mice. It was chosen as the experimental animal because its epidermis is thick enough to show whatever changes would take place.

The aim of this work is to study the effect of oral isotretinoin on the guinea pig skin. Histological and histochemical methods are used as tools of study. It is hoped that it will be possible to correlate between the present results and the reported changes in aging and photo aging of the human skin.

## 2. Materials And Methods:-

### Materials:

Twenty five female guinea pig (*Cavia sp.*) were used in this study, 15 of them were adult animals (about 6 months old) and the other 10 were old animals (2- 4 years old). They were grouped as follows:-

Group I: 5 adult control female guinea pigs.

Group II: 5 adult female guinea pigs treated with oral isotretinoin for one month.

Group III: 5 adult female guinea pigs treated with oral isotretinoin for one month followed by cessation of treatment for one month.

Group IV: 5 old control female guinea pigs.

Group V: 5 old female guinea pigs treated with oral isotretinoin for one month.

Isotretinoin powder (13- cis – retinoic acid) was dissolved in cedar wood oil. A human equivalent dose of 1 mg /kg body weight, calculated according to the body surface area of the guinea pig (Ghosh, 1971) was administered orally to the animals using small gastric tube.

### Methods:

#### I- Light Microscopy:

Animals of all groups were sacrificed by ether anesthesia and specimens were obtained from the Skin of the central part of the back. The specimens were fixed in 10% neutral buffered formalin, embedded in paraffin and cut at 5 – 7  $\mu$ m. in thickness. They were stained by the following histological and histochemical stains:-

1. Hx &E. stain for general histological structure.
2. Masson trichrome stain for collagen fibers.
3. Orcein stain for elastic fibers.
4. Gomori 's silver stain for reticular fibers.
5. PAS for carbohydrates in general.
6. Fontana – Masson silver stain for melanin granules.

All methods were applied according to Drury and Wallington, (1980).

In Hx &E stained sections biostatistical analysis was done for:

1. Epidermal and epithelial thickness of the Malpighian, granular and horny layers in two different fields of every animal in the same group.
2. Sebaceous gland size in two different fields from the back of each animal in the same group. Measurements were done using micrometer slide and a graduated eyepiece. The mean value were calculated and t- test was applied for each case.
3. Mitotic index was calculated by counting the number of mitotic figures per 100 resting cell in 5 different fields in all groups. The Mean values were calculated and the t- test was done.

## 3. Results

### Histology:

#### Control Adult Animals:

The epidermis of the back skin is composed mainly of a basal cell layer, 3-5 spinous cell layers, 1-2 granular cell layers and a thin keratinous layer. The dermal papillae are few and short. The basal layer is formed of tall columnar cells with oval vesicular nuclei and basophilic cytoplasm having numerous cytoplasmic extensions towards the dermis. The spinous cells are polyhedral with slightly basophilic cytoplasm and large rounded vesicular nuclei. The granular layer is formed of flattened cells filled with basophilic granules that mask their nuclei (Fig.1). Mitotic figures were commonly observed in the basal and spinous cells. Some clear cells were seen interposed among the keratinocytes. Some of these

cells were distinguished as melanocytes having dark smaller nuclei with numerous cytoplasmic extensions among the neighboring keratinocytes.

The dermis consists of a thin papillary layer and a thick reticular layer through which blood vessels, numerous lymphatics, hair follicles, sebaceous glands and arector Pilli muscles are present. Sweat glands were very seldomly seen (Fig. 1). Fine collagenous fibers passing parallel to the skin surface are present in the papillary dermis. Thick collagenous bundles running in different directions are present in the reticular dermis (Fig. 2). Numerous elastic fibers run in the dermis parallel to the skin surface in the form of long thin branched fibers occupying the papillary and the upper part of the reticular dermis (Fig.3). The basement membrane of the epidermis contains numerous short reticular fibers that are present also around the hair follicles and the sebaceous glands (Fig.4).

#### **Treated Adult Animals:**

Treatment of the adult female guinea pigs with isotretinoin for one month led to marked thinning of the cellular layers of the epidermis while the stratum corneum is thicker (Fig. 5-A). The decrease in the thickness of the Malpighian and the granular layer and the increase in the thickness of the stratum corneum were highly significant (Table 1). The basal cells are small with darkly stained nuclei. The spinous cells are 1-2 layers with rounded nuclei and acidophilic cytoplasm (Fig. 5-B). Mitotic figures were rarely observed among the keratinocytes. This decrease in mitotic activity is highly significant (Table 2). The stratum corneum appears as a thick loose layer of swollen corneocytes with thickened membranes (Figs.5-A&B). The most characteristic feature is that most of the hair follicles are devoid of hair shafts and the follicles are in the form of narrow canals consisting of one or two layers of small cuboidal or low columnar acidophilic cells and dark rounded or oval nuclei. In intact hair, they appear as masses of cells surrounded by thick C.T. sheath and cellular infiltration. The sebaceous glands are smaller in size compared to those seen in the adult control animals (Figs.5-A&B). Decrease in the amount of collagenous and elastic fibers was observed compared to those in the adult control group (Figs. 6&7).The elastic fibers are mainly confined to the outer dermal layer (Fig. 7). The amount of reticular fibers in the basement membranes surrounding the epidermis, the hair follicles and the sebaceous glands is increased in amount (Fig. 8).

After one month of cessation of treatment, the back skin appeared more or less similar to the adult control in its epidermal thickness (Fig. 9; Table 1), mitotic activity of the epidermal cells (Table 2),

sebaceous gland size (Fig. 9; Table 3) and in the density of C.T. fibers (Fig.10).

#### **Old Control:**

In old control female guinea pig, compared to the control adult animals, the epidermis is relatively thin but this thinning is not significant (Table 4). The basal layer consists of columnar cells with dark oval nuclei without visible cytoplasmic extensions. The spinous layer is 2-3 layers of polyhedral cells with small rounded nuclei which may be indented by cytoplasmic vacuoles (Fig.11A). Significant decrease in the mitotic activity was noticed compared to that of the adult control group (Table 5). The granular layer is 1-2 layers of flattened cells with relatively lesser amount of basophilic granules. The stratum corneum appears as loosely attached swollen corneocytes with thickened membranes (Figs. 11-A&B). The sebaceous gland are relatively smaller in size (Table 6).The collagenous fibers are less abundant (Fig.12) and the elastic fibers are shorter and less numerous (Fig.13) compared to those seen in the adult control animals. Reticular fibers are similar to those observed in the adult control group

#### **Treated Old Animals:**

Treatment of the old animals with isotretinoin for one month led to significant thinning of the epidermis especially of the cellular layers (Fig.14 A; Table 4). The spinous cells contain dense dark oval nuclei. The granular layer is discontinuous with few basophilic granules. The stratum corneum appears gelatinous and granulated (Figs. 14A&B).Mitotic figures were very rarely seen among the epidermal cells (Table 5). The size of the sebaceous glands was significantly decreased (Table 3). Also decrease in the amount of collagenous and elastic fibers was observed (Figs. 15&16). No significant change in the amount of reticular fibers was observed

#### **Histochemistry:**

##### **1- PAS method for carbohydrates:**

In the adult control animals, some epidermal cells show weak PAS positive reaction, while the basement membranes of the epidermis, hair follicles, sebaceous glands and C.T. dermis show a strong positive PAS reaction (Fig. 17). The intensity of the positive reaction was decreased after one month of treatment (Fig. 18).

After cessation of treatment, the intensity of the positive reaction became more or less similar to that of the control group.

In the old control animals, the C.T. dermis and the basement membranes are weak PAS positive (Fig.19). Moderate increase in the intensity of the PAS positive reaction was observed after one month of treatment (Fig. 20).

##### **2- Fontana Masson Silver Method For Melanin Granules:**

In the adult control animals, a considerable amount of melanin granules are present in the basal layer of the epidermis, fading gradually towards the stratum corneum (Fig. 21).

A marked increase in the amount of melanin granules was observed after one month of treatment with isotretinoin in all layers of the epidermis. The granules occupy the infranuclear region in the basal layer while they tend to occupy the supranuclear one in the successive layers (Fig. 22).

The density of the melanin granules is more or less similar to that of the control animals after cessation of treatment (Fig. 23).

In old control animals there are greater number of granules in the epidermal cells compared to that present in the adult control animals (Fig.24). An accumulation of a large number of the granules was observed after one month of treatment (Fig.25).

**Table(1): Thickness of the different layers of the epidermis of the back thin skin of different groups of adult animals.**

**I- Stratum Malpighi**

	Adult control	Adult treated for one month	After cessation
Mean	26.1 µm	18.9 µm	27.8 µm
±SD	3.9 µm	2.5 µm	2.7 µm
±SE	0.9 µm	0.6 µm	0.6 µm
P value	-----	0.0001**	0.07 N.S

**II- Stratum Granulosum**

	Adult control	Adult treated for one month	After cessation
Mean	5.9 µm	4.6 µm	6.2 µm
±SD	1.7 µm	1.8 µm	1.7 µm
±SE	0.4 µm	0.5 µm	0.4 µm
P value	-----	0.034*	0.73 N.S

**III- Stratum Lucidum and Corneum**

	Adult control	Adult treated for one month	After cessation
Mean	8.0 µm	12.9 µm	7.7 µm
±SD	1.2 µm	3.8 µm	1.7 µm
±SE	0.5 µm	0.9 µm	0.4 µm
P value	----	0.0001**	0.62 N.S

Notes: P value > 0.05 NS non significant. P Value ≤ 0.05 \* significant. P Value ≤ 0.01 \*\* highly significant

**Table (2): Mitotic % in the different groups of the adult animals in back thin skin epidermis.**

	Adult control	Adult treated for one month	After cessation
Mean	2.8	1.4	2.4
±SD	0.8	1.1	1.1
±SE	0.4	0.5	0.5
P value	----	0.003**	0.62 N.S

**Table (3): The size of the sebaceous glands of the different groups of the adult animals in the back thin skin.**

	Adult control	Adult treated for one month	After cessation
Mean	48.2 µm	32.7 µm	46.9 µm
±SD	14.2 µm	7.5 µm	11.8 µm
±SE	2.6 µm	1.4 µm	2.0 µm
P value	-----	0.0001**	0.68 N.S

Notes: P value > 0.05 NS non significant. P Value ≤ 0.05 \* significant. P Value ≤ 0.01 \*\* highly significant.

**Table (4): Thickness of the different layers of the epidermis of the back thin skin of different groups of old animals.**

**I- Stratum Malpighi**

	Adult control	old control	old treated for one month
Mean	26.1 µm	24.5 µm	13.8 µm
±SD	3.9 µm	2.5 µm	2.3 µm
±SE	0.9 µm	0.8 µm	0.5 µm
P value	-----	0.20 N.S	0.000001**

**II- Stratum Granulosum**

	Adult control	old control	old treated for one month
Mean	5.9 µm	6.3 µm	4.0 µm
±SD	1.7 µm	2.0 µm	1.8 µm
±SE	0.4 µm	0.5 µm	0.5 µm
P value	-----	0.63 N.S	0.005 **

**III- Stratum Lucidum and Corneum**

	Adult control	old control	old treated for one month
Mean	8.0 $\mu\text{m}$	7.0 $\mu\text{m}$	4.0 $\mu\text{m}$
$\pm\text{SD}$	1.2 $\mu\text{m}$	2.0 $\mu\text{m}$	1.8 $\mu\text{m}$
$\pm\text{SE}$	0.5 $\mu\text{m}$	0.5 $\mu\text{m}$	0.5 $\mu\text{m}$
<i>P</i> value	----	0.13 N.S	0.005 **

Notes: *P* value > 0.05 NS non significant. *P* Value  $\leq$  0.05 \* significant. *P* Value  $\leq$  0.01 \*\* highly significant.

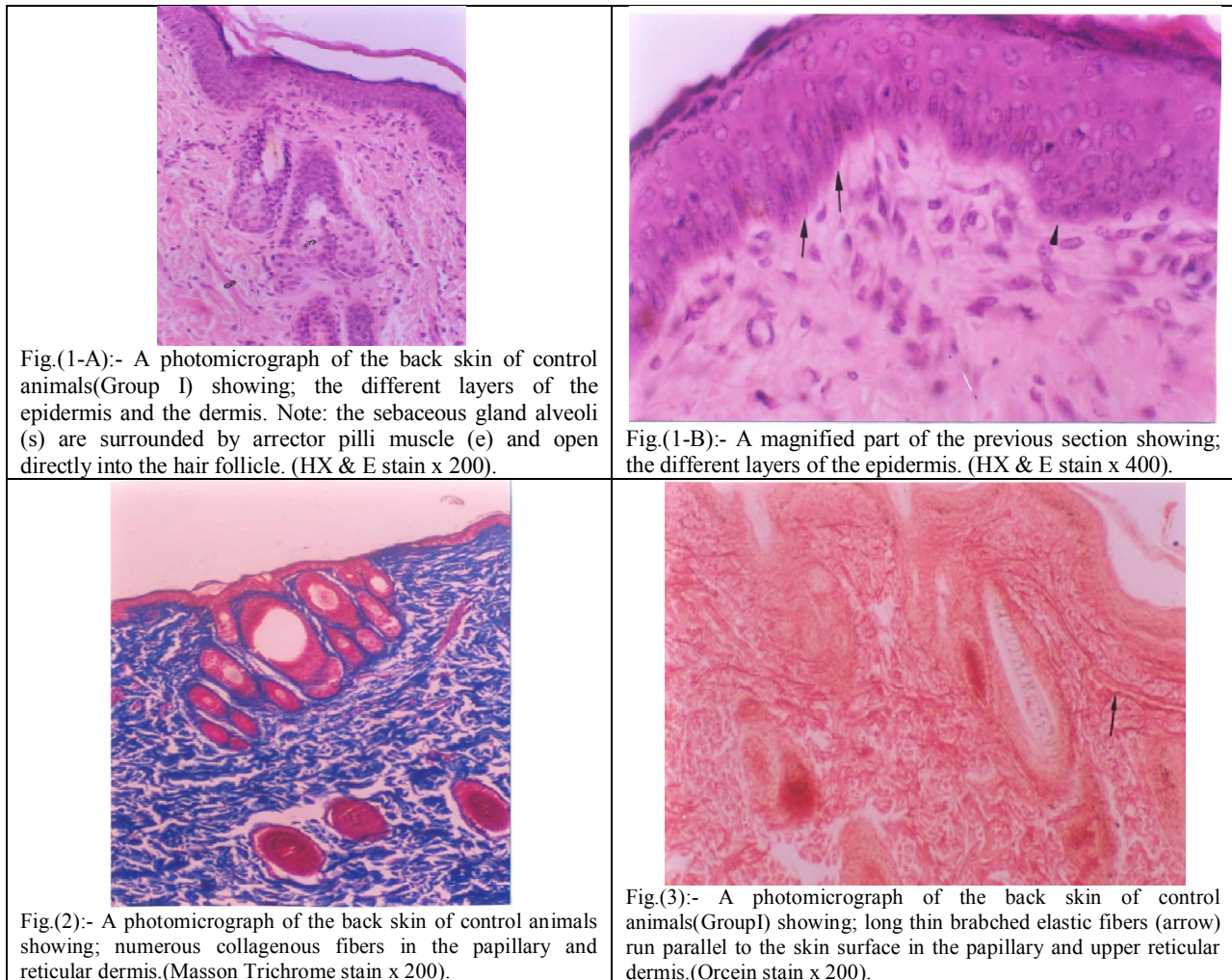
**Table (5): Mitotic % in the different groups of the old animals in back thin skin epidermis.**

	Adult control	old control	old treated for one month
Mean	2.8	1.0	0.4
$\pm\text{SD}$	0.8	0.7	0.5
$\pm\text{SE}$	0.4	0.3	0.2
<i>P</i> value	----	0.006**	0.1257 N.S

**Table (6): The size of the sebaceous glands of the different groups of the old animals in the back thin skin.**

	Adult control	old control	old treated for one month
Mean	48.2 $\mu\text{m}$	25.5 $\mu\text{m}$	11.9 $\mu\text{m}$
$\pm\text{SD}$	14.2 $\mu\text{m}$	6.0 $\mu\text{m}$	4.1 $\mu\text{m}$
$\pm\text{SE}$	2.6 $\mu\text{m}$	1.1 $\mu\text{m}$	0.8
<i>P</i> value	-----	0.0001**	0.0001**

Notes: *P* value > 0.05 NS non significant. *P* Value  $\leq$  0.05 \* significant. *P* Value  $\leq$  0.01 \*\* highly significant.



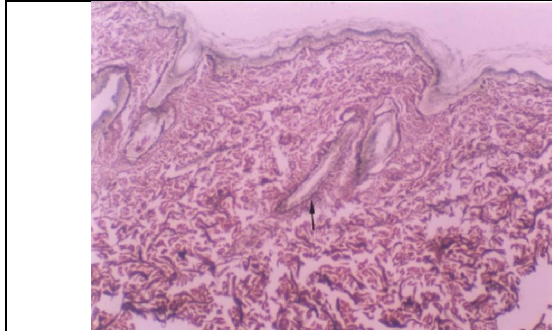


Fig.(4):- A photomicrograph of the back skin of control animals showing; very fine numerous reticular fibers in the basement membrane of the epidermis, the hair follicles and the sebaceous glands (arrow).(Gomor's reticulin stain x 200).

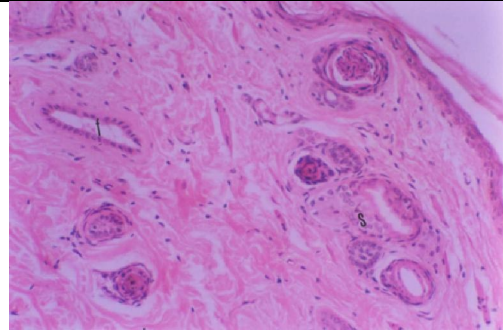


Fig.(5-A):- A photomicrograph of the back skin of Group II showing; thinning of the epidermis and the dermis, degenerated hair follicles (f) and small sized sebaceous gland alveoli (s). (HX & E stain x 200).

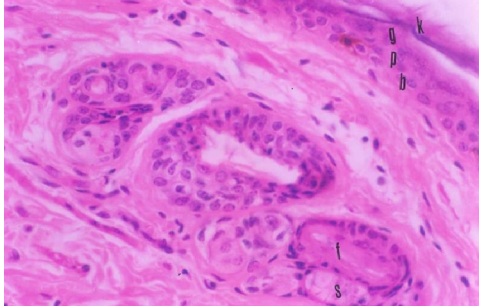


Fig.(5-B):- A magnified part of the previous section showing; thinning of the spinous layers of the epidermis (p), ill-defined granular layer (g), loose keratinous layer (k), degenerated hair follicles (f) with accompanying small sized sebaceous gland (s). (HX & E stain x 400).

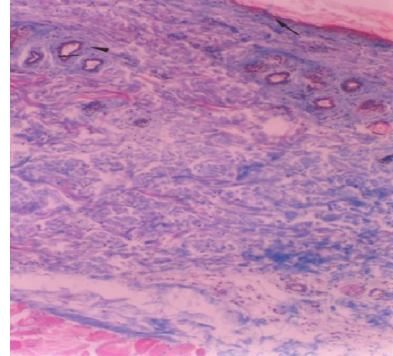


Fig.(6):- A photomicrograph of the back skin of Group II animals showing; decrease in the amount of collagenous fibers in the papillary and reticular dermis. (Masson Trichrome stain x 200).

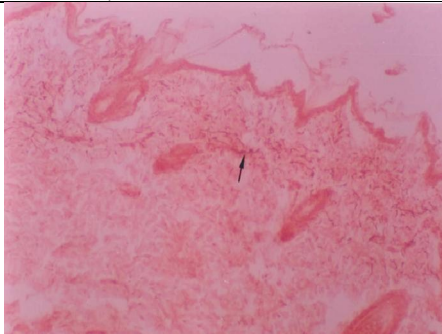


Fig.(7):- A photomicrograph of the back skin of Group II animals showing; decrease in the amount of elastic fibers (arrow) in the papillary and upper reticular dermis. (Orcein stain x 200).

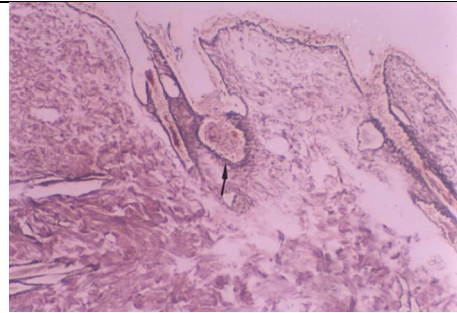


Fig.(8):- A photomicrograph of the back skin of Group II animals showing; increase in the amount of reticular fibers in the basement membrane of the epidermis, the hair follicles and the sebaceous glands (arrow). (Gomor's reticulin stain x 200).

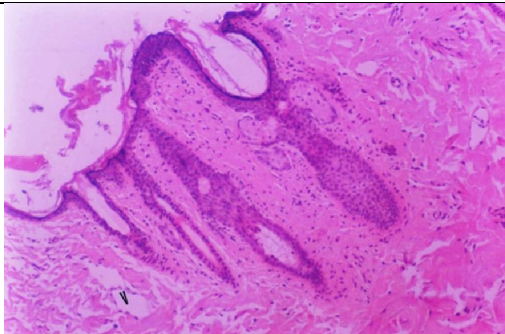


Fig.(9):- A photomicrograph of the back skin of Group III showing; the epidermis and the dermis appear more or less similar to that of the control animals. (HX & E stain x 200).

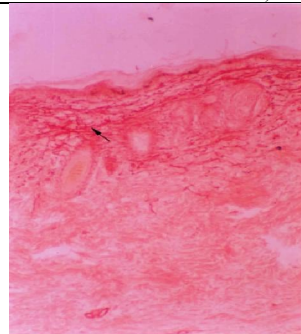


Fig.(10):- A photomicrograph of the back skin of Group III animals showing; numerous long thin and branching elastic fibers (arrow) in the papillary and upper reticular dermis. (Orcein stain x 200).

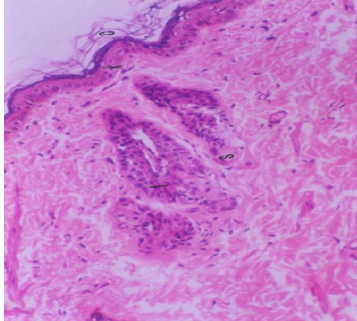


Fig.(11-A):- A photomicrograph of the back skin of Group IV showing; thin epidermis with vacuolated keratinocytes(k), loose stratum corneum(c), small sized sebaceous glands(s),and degenerated cells of the hair follicles(f). (HX & E stain x 200).

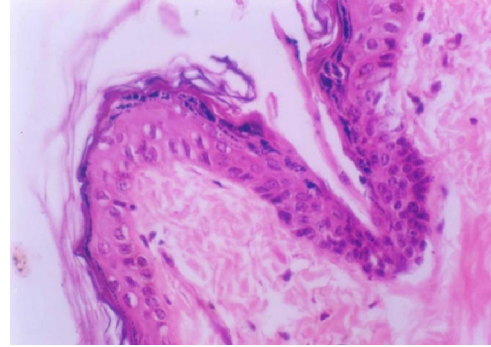


Fig.(11-B):- A magnified part of the previous section showing; thinning of the layers of the epidermis and very loose stratum corneum. (HX & E stain x 400).

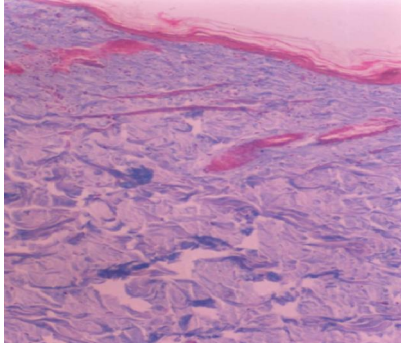


Fig.(12):- A photomicrograph of the back skin of Group IV animals showing; moderate amount of collagenous fibers in the papillary and reticular dermis. (Masson Trichrome stain x 200).

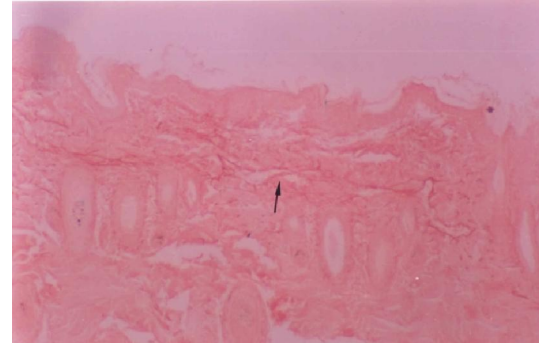


Fig.(13):- A photomicrograph of the back skin of Group IV animals showing; few amount of elastic fibers (arrow) in the papillary and upper reticular dermis. (Orcein stain x 200).

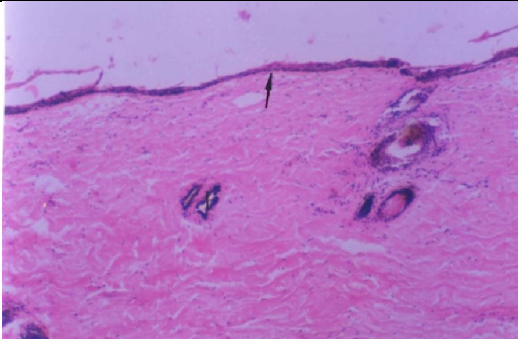


Fig.(14A):- A photomicrograph of the back skin of Group V showing; a marked thinning of the epidermis (arrow),and marked degeneration of the hair follicles(f). (HX & E stain x 200).

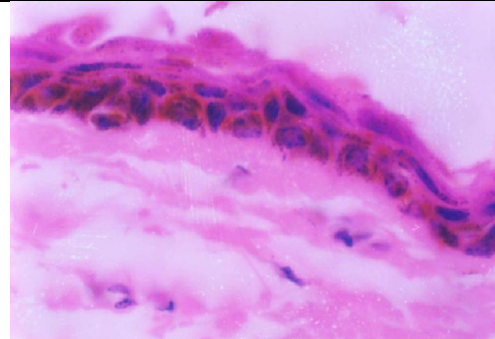


Fig.(14B):- A magnified part of the previous section showing; thinning of the layers of the epidermis. (HX & E stain x 400).

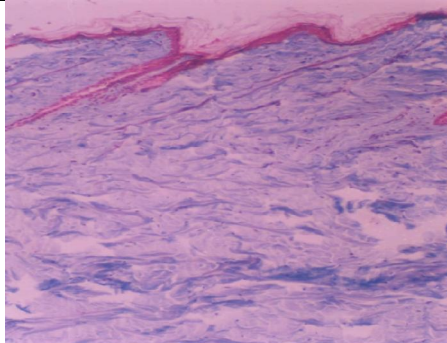


Fig.(15):- A photomicrograph of the back skin of Group V animals showing; the amount of collagenous fibers in the papillary and reticular dermis appeared more or less similar to the old control group. (Masson Trichrome stain x 200).

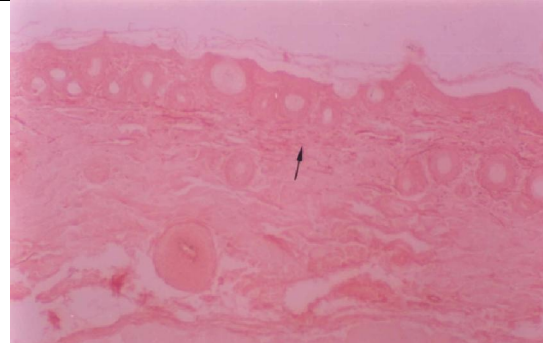


Fig.(16):- A photomicrograph of the back skin of Group V animals showing; marked decrease in the amount of elastic fibers (arrow) in the papillary and upper reticular dermis. (Orcein stain x 200).

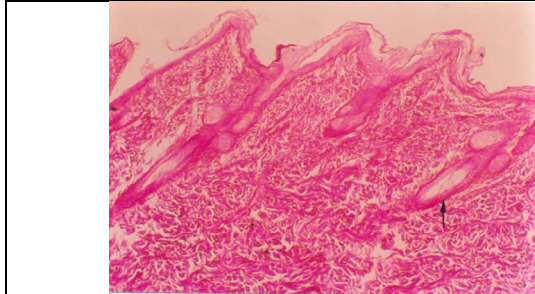


Fig.(17):- A photomicrograph of the back skin of Group I animals showing; strong PAS positive reaction was observed in the basement membrane of the epidermis, hair follicles and sebaceous gland. (PAS reaction x 200).

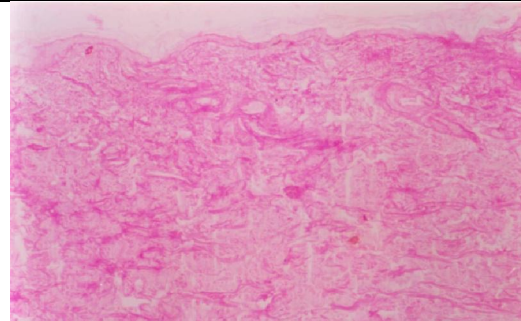


Fig.(18):- A photomicrograph of the back skin of Group II animals showing; marked decrease in the intensity of the PAS positive reaction. (PAS reaction x 200).

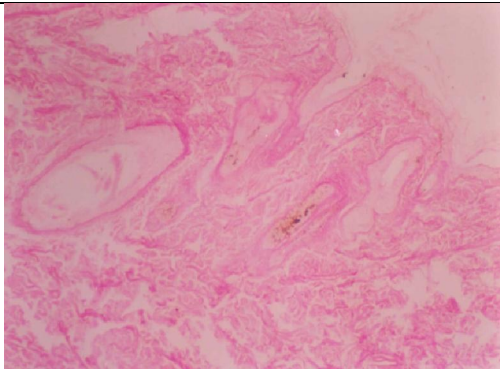


Fig.(19):- A photomicrograph of the back skin of Group IV animals showing; moderate PAS positive reaction. (PAS reaction x 200).

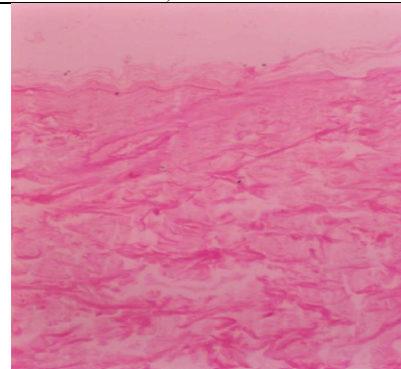


Fig.(20):- A photomicrograph of the back skin of Group V animals showing; moderate increase in the intensity of the PAS positive reaction. (PAS reaction x 200).

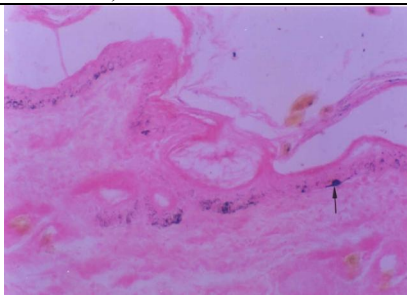


Fig.(21):- A photomicrograph of the back skin of Group I animals showing; some melanin granules in the epidermal cells. Note the branched melanocytes among the basal cells (arrow). (Fontana-Masson Silver x 200).

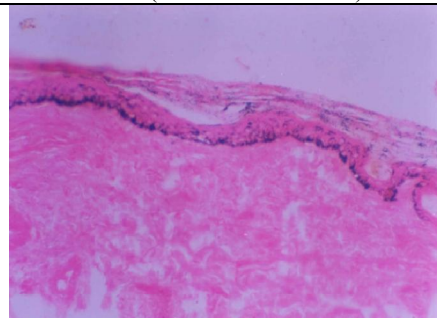


Fig.(22):- A photomicrograph of the back skin of Group II animals showing; numerous melanin granules in the epidermal cells. (Fontana-Masson Silver x 200).

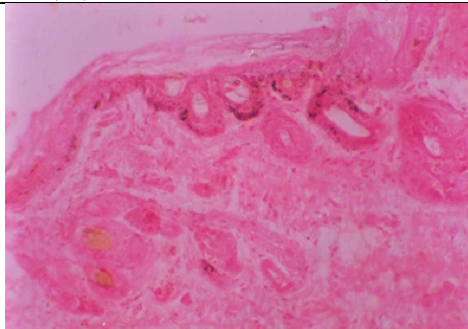


Fig.(23):- A photomicrograph of the back skin of Group III animals showing; the melanin granules in the epidermal cells appeared more or less similar to the adult control group. (Fontana-Masson Silver x 200).

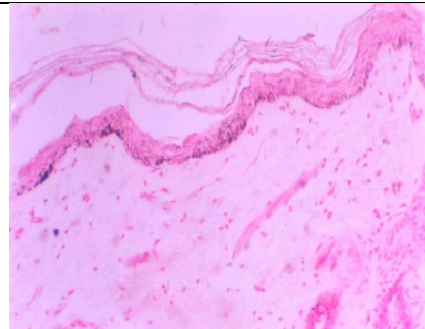


Fig.(24):- A photomicrograph of the back skin of Group IV animals showing; large amount of melanin granules in the epidermal cells compared to that of the adult control animals. (Fontana-Masson Silver x 200).



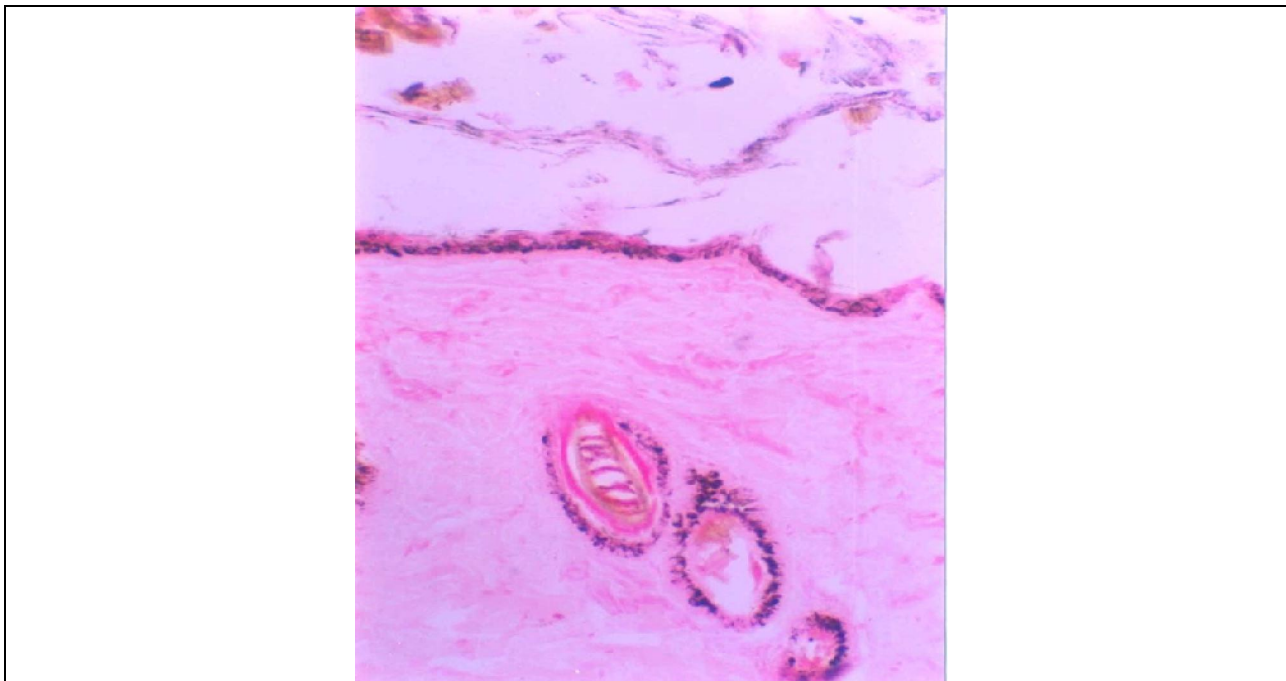


Fig.(25):- A photomicrograph of the back skin of Group V animals showing; increase in the amount of melanin granules in the epidermal cells compared to that of the old control animals. (Fontana-Masson Silver x 200)

#### 4. Discussion

The skin has been considered an epithelial tissue of particular interest as a target for the effects of vitamin A and other retinoids. This is because the structure and function of the skin is dependent on retinoid-influenced cellular division, differentiation and keratinization. Similarly, many of the physiological responses of the skin, such as dermal aging, immune defense, and wound healing, are significantly affected by retinoids (Fu *et al.*, 2007).

Retinoids have been widely used in the management of specific dermatological disorders such as acne vulgaris (Goswami, *et al.*, 1997) psoriasis, keratinization disorders and cutaneous malignancies (Moise *et al.*, 2007). In addition, they have been approved for treatment of photoaging and biologic skin aging (Sakuta and Kanayama, 2006). The role of vitamin A as a sunscreen that protects against ultraviolet light has also been suggested (Antille *et al.*, 2003).

In the present study, treatment of adult and old animals with biometric studies revealed decreased thickness of the cellular layers (stratum Malpighi and stratum granulosum) in thin skin of the back after one month of treatment with oral isotretinoin. The literature dealing with the effect of the therapeutic dose of oral isotretinoin on epidermal thickness is meager. Elias *et al.* (1981) and Tsambaos *et al.* (1980) found that oral isotretinoin in a dose of 50-100 mg/kg/day cause increased epidermal thickness in

hairless mouse and guinea pig respectively. Also Amany (2008) found highly significant increase ( $P < 0.001$ ) in the mean thickness of the epidermis in treated animals with topical application of 0.05% tretinoin cream when compared with the control group. This contradiction between these results and the results of the present study could be attributed to the much dose used in the previous studies. It was found that the effect of vitamin A is dependent on a number of variables such as the dose, the duration of exposure, the chemical form of vitamin A used, species differences and regional differences in the same species (Logan, 1972). The increase in the thickness of stratum corneum in some areas can be explained by the loosening and fraying of this part of the epidermis which is obvious in the histological sections. This loosening was also previously reported in hairless mouse after oral isotretinoin therapy (Elias *et al.*, 1981).

The significant decrease in the mitotic figures in treated animals is in accordance with the result of Pinkus and Hunter (1964) who noted a decrease in the number of mitotic figures after oral administration of vitamin A. Also, an increase in the epidermal transit time in guinea-pig skin under the influence of tretinoin has been documented (Christopher's and Braun-Falco, 1970). These changes in the mitotic figures are most probably due to the anti-proliferative effect of isotretinoin. On the contrary, topical application of retinoic acid was proven to increase mitotic figures in

the skin of human (Griffiths *et al.*, 1993), guinea pig ear skin (Aso *et al.*, 1976) and rats (Amany, 2008).

Oral isotretinoin leads to distinct signs of intoxication in guinea pig epidermis, such as vacuolar cytoplasmic disintegration, nuclear pyknosis and destructions and spongiosis of the cells. Cytoplasmic disintegration of the keratinocytes is most probably due to the activation of lysosomal enzymes in the epidermis of the treated animals. Studies with vitamin A and retinoic acid have revealed that these compounds are potent lysosomal labializes (Jarrett *et al.*, 1978 and Amany, 2008). It is possible therefore, that isotretinoin may affect epidermal lysosomes, leading to autolytic processes in the viable epidermis. The inhibitory effect of vitamin A on epidermal differentiation provides the basis for the therapeutic uses of retinoids in skin conditions characterized by hyper keratinization (Tsamboas *et al.*, 1980).

Nelson *et al.* (2006) reexamine the effects of isotretinoin, alitretinoin, and tretinoin on cell proliferation, cell cycle proteins, and apoptosis of SEB-1 sebocytes and keratinocytes and confirm the previous reports concerning the dose- and time dependent antiproliferative effect of isotretinoin on natural and immortalized SZ95 sebocytes (Zouboulis *et al.*, 1991; Zouboulis *et al.*, 1999; Tsukada *et al.*, 2000). A portion of this decrease was attributed to an influence on the G1/S phase of the cell cycle, as evidenced by decreased DNA synthesis, increased p21 protein, and decreased cyclin D1 protein, and, therefore, to cell cycle arrest.

By using annexin V-FITC staining, TUNEL staining, and cleaved caspase 3 protein analysis, Nelson *et al.* (2006) were, however, able to detect a marginal induction of apoptosis in SEB-1 sebocytes by isotretinoin after 48 and 72 hours of treatment. The ability of isotretinoin to induce apoptosis was not recapitulated by alitretinoin or tretinoin. The induction of cell cycle arrest and apoptosis by isotretinoin was specific to sebocytes, as the compound failed to induce apoptosis in HaCaT keratinocytes or normal human epidermal keratinocytes.

Retinoids have been shown to block terminal epidermal differentiation and to induce epidermal and stratum corneum loosening which may, in turn, lead to loss of epidermal cohesion and abnormal barrier function. On the ultrastructural level, retinoids were shown to induce active shedding of desmosomes by the epidermal cells resulting in many fewer attachment points along the cell membranes of the outer epidermis. Loss of desmosomes, coupled with decreased tonofilaments, enhanced keratinocyte autolysis and the generation of less mature keratins cause loosening and fragility of the stratum corneum, the so-called anti-keratinizing effects (Eichner *et al.*, 1996).

Topical tretinoin appears to stimulate melano genesis in pigs. By E.M the number and size of melanocytes in tretinoin-treated areas of the skin are greatly increased. The melanosomes are larger and more abundant in the melanocytes and keratinocytes (Zheng and Klingman, 1991). These results are similar to those obtained in the present study. The increase in the density of melanin granules in the epidermal cells of guinea pig skin may be explained also by the same theory of that vitamin A is of direct effect of the lysosomal enzymes. Isotretinoin, therefore may activate tyrosinase enzyme by the same mechanism mentioned above causing increased production of melanin granules. In contrast, many authors stated that topical retinoids improve the pigmented lesions in photo aged skin in a dose dependent manner (Bhawan *et al.*, 1991; Weinstein *et al.*, 1991). Also, clinical trials have shown that topical tretinoin can modify dysplastic or malignant melanocytes. Fading of some lesions or the elimination of some dysplastic nevi has been reported (Edwards and Jaffe, 1990).

Retinoic acid increased tyrosinase activity and melanin synthesis in melanoma cell lines (Edward *et al.*, 1988). Another study has demonstrated that, in melanoma cell lines, acitretin enhanced melanin synthesis while other reinoids, isotretinoin and temarotene have no significant effects on melanin production (Garbe *et al.*, 1991). An inhibition of Milano genesis has also been demonstrated in some melanoma cell lines (Chakraborty *et al.*, 1990). Few data are available on the effect of retinoic acid on normal human melanocytes. In a review of Yaar and Gilehrest (1991), it was stated that retinoid had not been demonstrated to have any effect on these cells.

There is significant decrease in the size of the sebaceous glands in the treated thin skin. This result is in accordance with the results of previous investigators who showed that oral retinoid decrease the size of the sebaceous glands in the humans (Gomez, 1982; Plewig *et al.*, 1982). Also, topical tretinoin decrease the size of the sebaceous glands and suppress sebum production in rats (Amany, 2008). Isotretinoin has a potent inhibitory effect on both cell proliferation and lipid synthesis in human sebocytes *in vitro* (Zouboulis *et al.*, 1991). These effects are most probably due to the anti-proliferative activity of isotretinoin resulting in prolongation of maturation of basal sebocytes and this explains the potent effect of isotretinoin in clinical treatment of hyperactive seboaceous glands in acne vulgaris.

In the present study, significant decrease in the amount of collagen fibers was demonstrated in treated adult and old animals. Many investigators reported that various retinoid have inhibitory effect on type I and/or III collagen synthesis by cultured human fibroblasts (Osada *et al.*, 1994; Shigematsu and

Tajima, 1995). Also retinoid inhibit synthesis of collagen type II by cultured rabbit chondrocytes (Benya and Padilla, 1986). The *in vivo* effect of retinoid on the collagen fiber synthesis was conflicting. Some authors proved that topical retinoid stimulate their synthesis in human (Kligman *et al.*,1999) and in the experimental animals (Schwartz *et al.*,1991). Others claimed that topical tretinoin in human does not induce de novo synthesis of collagen and it does not counteract the inhibitory effect of a potent corticosteroid on collagen pro peptides (Haapasaari *et al.*,1997), while others proved that topical retinoid could prevent the inhibitory effect of concomittent corticosteroids. The literatures dealing with the effect of oral retinoid on dermal collagenous fibers are few. Some authors reported that no dermal abnormalities were found either by L.M or E.M in human and hairless mice receiving oral isotretinoin and etretinate. Oral etretinate administration proved to have an inhibitory effect on collagen synthesis by fibroblasts as the serum carboxyterminal pro peptide of type I pro collagen level is greatly decreased (Osada *et al.*,1994).

The mechanism of the inhibitory effect of retinoid on collagen synthesis probably occurs due to the reduction in the pro collagen m RNA (Oikarinen *et al.*, 1989). Retinoid increase lytic effects of lysosomal enzymes of which collagenase are one.

In the present study, the reticular fibers are increased in treated adult and old animals. These fibers are considered as immature collagen fibers. This increase in the amount of reticular fibers which are mainly formed of collagen III may compensate the decrease in the mature collagen fibers which are formed of collagen I,II and III.

The most characteristic effect of isotretinoin on thin skin is the degenerative changes that occur in the hair follicles. Most of these follicles were transformed into thin cellular canals consisting of a few flattened cells surrounded by mononuclear cell infiltration. This degeneration explains the easily removable hair and alopecia that occurred in some areas in treated animals. Similar degenerative changes in the hair follicles were described in other cases of alopecia as in hot comb alopecia described by Sperling and Sau (1992). Also this appearance resembles hair follicles in the catagon phase. This result confirms that the anti-proliferative effect of isotretinoin on hyperactive proliferating hair matrix cells responsible for regeneration of hair follicles that occur in normal hair growth cycle.

In conclusion, the long-term application of isotretinoin may affect the normal structure of the epidermis and dermis being partially reversible after withdrawal of the drug. Due to its side-effects, the use,

dosage, and time frame of tretinoin should be restricted to the advice of dermatologists.

The general decrease in growth, proliferation and differentiation of the guinea pig dermal and epidermal cells demonstrated in this study may be explained by the result obtained by Fisher *et al.* (1992) in that retinoic acid decreased intracellular expression of transforming growth factors  $\beta$ . These are multifunctional peptides that exert potent regulatory effects during embryogenesis and participate in the regulation of growth and differentiation. This means that retinoic acid decrease that factor in cells capable of high proliferative activity in postnatal life resembling the hyper-proliferative embryonic cells. Of these cells are the keratinocytes, basal undifferentiated cells of the sebaceous glands, the hair matrix cells, and the dermal cells, mainly, the fibroblasts. Thus this anti-proliferative effect of isotretinoin, together with its anti-differentiating effect is responsible for most of demonstrated effects of isotretinoin in this study. Retinoids taken by mouth may cause birth deformities (Chaudhari *et al.*,2011).

It is therefore recommended that retinoid in all forms may not be used by pregnant women because of the possible effect of that drug on the activity of proliferating and differentiating embryonic cells.

## References

1. Fu PP, Xia Q, Boudreau MD, Howard PC, Tolleson WH and Warner WG (2007): Physiological role of retinyl palmitate in the skin. *Vitam. Horm.*; 75: 223-256.
2. Goswami BC, Baishya B, Barua AB and Olson JA (1997): Topical retinoyl beta-glucuronide is an effective treatment of mild to moderate acne vulgans in Asian-Indian patients. *Skin Pharmacol.Appl.Skin Physiol.* Jul-Aug; 12(4): 167-173.
3. Moise AR, Noy N, Palczewski K and Blaner WS (2007): Delivery of retinoid-based therapies to target tissues. *Biochemistry Apr* 17; 46 (15): 4449-4458.
4. Sakuta T and Kanayama T (2006): Marked improvement induced in photoaged skin of hairless mouse by ER36009, a novel RARgamma-specific retinoid, but not by ER35794, an RXR-selective agonist. *Int. J. Dermatol. Nov*; 45 (11): 1288-1295.
5. Antille C, Tran C, Sorg O, Carraux P, Didierjean L and Saurat JH (2003): Vitamin A exerts a photoprotective action in skin by absorbing ultraviolet B radiation. *J.Invest. Dermatol. Nov*; 121 (5): 1163-1167.
6. Kligman AM, Pagnoni A and Stoudermayer T (1999): Topical retinol improves cellulite. *J. Dermatol. Treat.*; 10: 119-125
7. Amany M. Mousa(2008):Histological and Immunohistochemical Study on the Effect of Tretinoin on the Thin Skin of Adult Male Albino Rat. *Egypt. J. HistoL Vol. 31, No.2, Dec. 2008: 208*
8. Eichner R, Gendimenico GJ, Kahn M, Mallon JP, Capetola RJ and Mezick JA (1996): Effects of long-term retinoic acid treatment on epidermal differentiation *in vivo*: Specific modifications in the programme of terminal differentiation. *Br.J.Dermatol. Nov*; 135 (5): 687-695.
9. Chaudhari P.M; Kawade P.V; and Funne S.M (2011): COSMECEUTICALS - A REVIEW. *International Journal Of Pharmacy& Technology*, Vol. 3 | Issue No.1 | 774-798.
10. Serri R, Iorizzo M. Cosmeceuticals(2008): focus on topical retinoids in photoaging. *Clin Dermatol.* 26(6):633-5.

11. Stratigos AJ, and Katsambas AD. (2009): The role of topical retinoids in the treatment of photoaging. *Dermatol.*; 27(2):217-24.
12. Elias,P.M. (1981): Epidermal lipids, membranes and keratinization. *Int.J. Dermatol.* 20:1-19.
13. Tsambaos,D.;Mahrle, G. and Orfanos, C.E.(1980): Epidermal changes induced by oral excess of aromatic retinoid in guinea pigs. *Arch. Dermatol.*267: 141- 152.
14. Logan, W.S. (1972): Vitamin A and keratinization. *Arch. Dermatol.* 105: 748-753.
15. Jarrett,A.; Wrench, R. and Mahmoud, B.L. (1978): Effects of retinyl acetate on epidermal proliferation and differentiation. Induced enzyme reactions in the epidermis.*Clin. Exp. Dermatol.*3: 173 178.
16. Griffiths, C.E.M.; Finkel, L.J. and Tranfaglia, M.G. (1993): An *in vivo* experimental model for effects of topical retinoic acid in human skin. *Br.J. Dermatol.*129: 389 – 394.
17. Christophers, E. and Braun- Falco,O. (1970): Mechanisms of parakeratosis. *Br.J.Dermatol.* 82: 268 – 276.
18. Pinkus, H. and Hunter, R. (1964): Biometric analysis of the effect of oral vitamin A on human epidermis. *J. Invest. Dermatol.*42: 131- 136.
19. Zheng, P. and Kligman, A.M.(1991): Topical tretinoin stimulates melanogenesis in the Yucatan micropig (abstract). *J. Invest. Dermatol.* 96: 576 – 579.
20. Yaar, M. and Gilchrist, B.A. (1991): Human melanocyte growth and differentiation: a decade of new data. *J. Invest. Dermatol.* 97: 611- 617.
21. Zouboulis, C.C.; Korge,B. and Akamatsu,H. (1991): Effects of 13-*cis*- retinoic acid, all- *trans*- retinoic acid, and acitretin on the proliferation, lipid synthesis and keratin expression of cultured human sebocytes *in vitro*. *J.Invest.Dermatol.*96: 792- 797.
22. Weinstein, G.D.; Nigra, T.P. and Pochi, P.E. (1991): Topical tretinoin for treatment of photodamaged skin: a multicenter study. *Arch. Dermatol.* 127: 659- 665.
23. Vora, M. and Karasek, M.A. (1994): Retinoids upregulate phagocytosis by human dermal microvascular endothelial cells. *J.Cell Physiology.* 159(3): 450- 456.
24. Garbe, C.; Krasagakis, K.; Kruger, S. and Orfanos,C.E. (1991): Potential of different retinoids to induce cellular differentiation of melanoma cells cultured *in vitro* (abstract). *J.Invest. Dermatol.* 96: 1000-1010.
25. Gendimenico, G.J. and Mezick, J.A. (1993): Pharmacological effects of retinoids on skin cells. *Skin Pharmacology.* 65(1): 24- 34.
26. Gomez, E.C.(1982): Actions of isotretinoin and etretinate on the pilosebaceous unit. *J.Am. Acad. Dermatol.* 6: 746 – 750.
27. Benya, P.D. and Padilla, S.R.(1986): Modulation of the rabbit chondrocyte phenotype by retinoic acid terminates type II collagen synthesis without inducing type I collagen: the modulated phenotype differs from that produced by subculture. *Dev.Biol.* 118: 296 – 305.
28. Bhawan, J.; Gonzalex-serve, A. and Nehal, K. (1991): Effects of tretinoin on photodamaged skin: a histologic study. *Arch. Dermatol.* 127: 666- 672.
29. Edward, M.; Gold, J.A. and Mackie, R.M. (1988): Different susceptibilities of melanoma cells to retinoic acid – induced changes in melanotic expression. *Biochem. Biophys.Res. Commun.* 155: 773- 778.
30. Edwards, L. and Jaffe, P.(1990): The effect of topical tretinoin on dysplastic nevi. *Arch. Dermatol.*126: 494- 499.
31. Chakraborty, A.; Orlow, S.J. and Pawelek, J.M.(1990): Stimulation of the receptor for melanocyte – stimulating hormone by retinoic acid. *FEBS Lett.* 276: 205- 208.
32. Osada, K.; Seishima, M. and Mori, S.(1994): Etretinate administration reduces serum propeptide of type I procollagen level in patients with psoriasis. *Br.J.Dermatol.* 131: 655- 659.
33. Plewig, G.; Nikolowski, J. and Wolff, H.H.(1982): Action of isotretinoin in acne rosacea and gram-negative folliculitis. *J. Am.Acad. Dermatol.*6: 766- 785.
34. synthesis and cell proliferation by retinoids in human skin fibroblasts. *J. Dermatol.Sci.* 9(2): 142 – 145.
35. Schwartz, E.; Cruickshank, F.A.; Mezick, J.A. and Kligman, L.H.(1991): Topical all-*trans* retinoic acid stimulates collagen synthesis *in vivo*. *J. invest. Dermatol.* 96: 975 – 978.
36. Sperling, L.C. and Sau, P. (1992): The follicular degeneration syndrome in black patients. *Arch. Dermatol.* 128 (1): 68 – 74.
37. Fisher, G.J.; Tavakkol, A. and Griffiths, C.E.M. (1992): Differential modulation of transforming growth factor B1 expression and mucin deposition by retinoic acid and sodium lauryl sulfate in human skin. *J.Invest. Dermatol.* 98 (1): 102- 108.
38. topical tretinoin on non- sun- exposed human skin connective tissue: induction of tenascin but no major effect on collagen metabolism. *Br.J. Dermatol.* 136: 891- 900.
39. Oikarinen, A.; Auorio, E. and Vuorio, T. (1989): Comparison of the effects of dexamethasone and 13- *Cis* – retinoic acid on connective tissue biosynthesis in human skin fibroblasts. *Arch. Dermatol.Res.*281: 273- 278 41- Drury, R.A.B. and Wallington E.A. (1980): "Carleton's Histological Technique, 5<sup>th</sup> ed. Oxford University, Press., Oxford, New York.
40. Aso, K.; Rabinowitz, I. and Farber, E.M. (1976): The role of prostaglandin E, cyclic AMP and cyclic GMP in the proliferation of guinea pig ear skin stimulated by topical application of vitamin A acid. *J.Invest. Dermatol.*67(2): 231- 234.
41. - Nelson AM, Gilliland KL, Cong Z, Thiboutot DM (2006) 13-*cis* Retinoic acid induces apoptosis and cell cycle arrest in human SEB-1 sebocytes. *J Invest Dermatol* 126:2178–2189
42. Zouboulis CC, Korge B, Akamatsu H, Xia L, Schiller S, Gollnick H *et al.* (1991) Effects of 13-*cis*-retinoic acid, all-*trans*-retinoic acid, and acitretin on the proliferation, lipid synthesis and keratin expression of cultured human sebocytes *in vitro*. *J Invest Dermatol* 96:792–7.
43. Zouboulis CC, Seltmann H, Neitzel H, Orfanos CE (1999) Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95). *J Invest Dermatol* 113:1011–20.
44. Tsukada M, Schröder M, Roos TC, Chandraratna RAS, Reichert U, Merk HF *et al.* (2000) 13- *cis* Retinoic acid exerts its specific activity on human sebocytes through selective intracellular isomerization to all-*trans* retinoic acid and binding to retinoid acid receptors. *J Invest Dermatol* 115:321–7.

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