

# Histomorphometric and Immunohistochemical Characteristics of the Skins of Egyptian Water Buffalo (*Bubalus bubalis*) and One-Humped Camel (*Camelus dromedarius*)

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With 4 figures & 4 tables

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## Abstract

The current work focuses on studying the histomorphological characterizations of the skin of Egyptian water buffaloes (*Bubalus bubalis*) and one-humped camels (*Camelus dromedarius*). Therefore, few in depth histological, morphometric and immunohistochemical comparative studies are so far available. This study was carried out on the skin specimens of fourteen adult, male Egyptian water buffaloes and one-humped camels (seven/each). Buffalo's skin showed several histological similarities with that of camel, but there were some histomorphometric and immunohistochemical differences. In spite of being the epidermis of buffalo significantly thicker than in camel, the horny layer of camel's skin was sig-

nificantly thicker than buffalo, in addition, it appeared laminar and weavy, while that of buffalo appeared compact. Hair follicles were more numerous in camel than in buffalo while sweat glands had larger diameter in buffalo. The secretory epithelium of the sweat glands varied from cuboidal with central nuclei in buffaloes to columnar with basal nuclei in camels. Immunohistochemically, vimentin and melanosome were over-expressed in camels than buffaloes. It is concluded that buffalo's skin was thicker than camel, which put it up for the production of skin quality requirements of the leather industry. However, higher vimentin content in camel skin may suggest higher tensile strength than in buffalo. Furthermore, it is reported that over-

expression of melanosome in camel's skin than buffalo may play a role in photoprotection against harmful sunny rays in harsh hot climate of the desert.

**Keywords:** Skin; histomorphometry; immunohistochemistry; buffalo; camel.

## Introduction

Skin is the greatest body organ, accounting for 15 – 20% of the total body weight. It performs a wide variety of fundamental functions likewise; protection against pathogens and harmful poisons, immunological defense, thermoregulation, waterproof, excretion of wastes, sensation and absorption of certain drugs. Moreover,—skin of animals as by-product has economic importance as a basic raw material in leather industries (Ozfiliz et al., 2012). Leather and leather goods industry play an important economic role for a lot of industrial countries all over the world.

In Egypt, skins of both Egyptian water buffaloes (*Bubalus bubalis*) and one-humped camels (*Camelus dromedarius*) are of the most commonly used for leather industry. Although buffalo's skin has high economic values (Said, 2011), until now in several regions, it has not been widely used as an industrial raw material for the purposes of leather

goods, but more practically used only as a raw material for the manufacture of leather materials of a parchment (Djojowidagdo, 1988) and skin crackers (Said, 2012). On the other hand, camel is considered as an important source of Egyptian income where it provides about 1300 tons of hides (Sooud, 1995).

Histologically, the skin is formed from two main layers; superficial epidermis of multilayer keratinocytes (Junqueira and Carnerio, 2003) and deep dermis which is subsequently divided into papillary and reticular connective tissue layers (Mobini, 2012). The dermis contains hair follicles and skin glands; sweat and sebaceous glands that are derived from epidermis-dermis interaction (Widelitz et al., 1997).

However, epidermis, dermis and skin appendages differ in their structures and features depending upon animal species and habitats (Dellmann, 1993) therefore, our study aimed to assess the histological, morphometric, and immunohistochemical characterizations of the skin in Egyptian water buffaloes (*Bubalus bubalis*) and one-humped camels (*Camelus dromedarius*) to reveal their environmental adaptation and their possible economic importance.

## Materials and Methods

### Animals

The current study was carried out on the skin of seven adult, male Egyptian water buffaloes (*Bubalus bubalis*) and seven one-humped camels (*Camelus dromedarius*). Different small specimens of skin were obtained from mid of the back of each animal (where the back of the animal is the largest part of the skin and the most exposed area to environmental conditions). All specimens were freshly collected directly within thirty minutes after slaughtering from Al-Warrak abattoir, El-Giza Governorate, Egypt. The animals were apparently healthy and free from any parasites and lesions. They were slaughtered according to the regulations of the Egyptian Abattoirs.

### Tissue processing

The specimens were immersed directly in 10% neutral buffered formaldehyde for one week. Following fixation, the specimens were preserved in 70% ethyl alcohol for about one week. The preserved specimens were then briefly rinsed into a graded series of ethanol (70%, 80%, 90% and absolute ethanol) at eight hours interval. The specimens were cleared in three changes of xylene and then embedded in paraffin wax. The paraffin blocks were transversely sectioned at 5 – 7  $\mu$ m thickness for

histological, morphometric and immunohistochemical studies.

### Histological procedures

The prepared paraffin sections were subjected to haematoxylin and eosin (H&E), Crossman's trichrome and periodic acid Schiff (PAS) technique. The afore-mentioned histological and histochemical techniques were followed as outlined after, Bancroft and Gamble (2008).

### Histomorphometry

A quantitative measurements included the thickness of skin epidermis, as well as, papillary and reticular layers was conducted using a computerized light microscope (Leica) and morphometric software of Leica application suite version 4.0 (LAS V4.0). At 40X objective lens, five different H&E stained sections were measured in ( $\mu$ m) for each animal. The values for each animal were averaged and compared to each other using student's *t*-test.

### Immunohistochemical procedures

The paraffin sections were deparaffinized and then rehydrated. The hydrated sections were subjected to preheated target retrieval solution (DakoCytomation, Carpinteria, CA, USA) for 40 min at 95 °C. The sections were then incubated with the primary antibodies for 1 h at room temperature (RT) after reducing the activity of endogenous peroxidase by

incubation of the sections in 3% hydrogen peroxide in methanol for 10 min. Sections were incubated with primary antibodies for 60 min at RT. Mouse anti-vimentin (Cat. # MS-129-P0, Thermo Fisher Scientific, Fer-mont, CA, USA) and mouse anti-melanosome (Cat. # 61-0042, Genemed Biotechnologies Inc, South San Francisco, CA, USA) were used at dilutions of 1:100 and 1:50 for demonstration of vimentin and melanosome respectively. Sections were incubated with anti-mouse IgG (1:200 dilution) as secondary antibody then, visualized using Vectastain<sup>®</sup> Elite reagent (Vector Laboratories, Burlingame, CA, USA) for 30 min. Sections were treated with liquid diaminobenzidine (DAB) (Dako Cytomation, CA, USA) for 5 min, counterstained with hematoxylin, then mounted and covered with coverslips. For negative controls, the primary antibodies were eliminated and exchanged with normal mouse IgG (Santa Cruz Biotechnology, CA, USA). The specificity of the immunoreactivities was confirmed by the absence of the staining in the negative control sections.

### **Qualitative evaluation of immunostaining**

The intensity of vimentin and melanosome immunostainings was scored according to Spencer and Bazer (1995). Intensities were classified as follows: negative (-)

when the cells had no any detectable immunostaining, weak (+), moderate (++) , and strong (+++). Three different slides per antibody for each individual animal were blindly examined at 40X objective lens.

### **Statistical analysis**

Two-tailed student's *t*-test was used to compare the quantitative data concerning the skin of both buffalo and camel.  $P < 0.05$  was considered statistically significant.

## **Results**

### **Histomorphometric observations**

There was no significant variation in histological structure of the skin from the different parts along the mid of back of the same species, and the major features are illustrated in Fig (1).

The skin of buffalo (Figs. 1A, E) and that of camel (Figs. 1B, F) was a vascular and highly cellular in the uppermost layer; the epidermis, while fibrous and highly vascular in the deeper layer; the dermis. Generally, the epithelocytes forming the epidermis was arranged into four strata (basalis, spinosum, granulosum, and corneum). The startum basalis composed of single layer of cuboidal cells resting on weavy basement membrane. Stratum spinosum was characterized by presence of several layers of large po-

lygonal cells. Meanwhile, stratum granulosum was differentiated into two to three layers of flattened squamous cells. The outermost layer, stratum corneum (horny layer), showed dead keratinized layers of scale like cells. In spite of the significantly ( $P < 0.05$ ) thick epidermis of the buffalo than that of the camel (Table 1), the stratum corneum of camel's skin is significantly ( $P < 0.05$ ) thicker than that of the buffalo (Table 2). The horny layer of the camel epidermis appeared to be laminar and weavy, while that of the buffalo was compact structures. However, horny layers of both buffalo and camel showed intense PAS positive reaction (Figs 1C, D).

Dermis of both buffalo (Figs 1A, E) and camel (Figs 1B, F) consisted of highly vascular superficial papillary layer which had fine pronounced collagen fibers with abundant fibroblasts, and deep reticular layer which had larger, denser loosely arranged collagen fibers with few fibroblasts. Skin appendages including hair follicles, sweat and sebaceous glands were located among the reticular layer of the dermis. Hair follicles were distributed singly or in groups but they are more numerous in camel than in buffalo. Sebaceous glands were simple, branched alveolar and commonly associated with hair follicles but occasionally found alone. Sweat glands were identified in both animals and present in the middle of

deep dermis (Figs 1E, F) they tend to be deeper in the camel dermis.

The glandular portion of the sweat glands was usually coiled tubular with secretory epithelium varying from low cuboidal in buffalo (Fig 1G) and columnar in camel (Fig 1I) with acidophilic cytoplasm and round central nuclei in buffalo and basally located nuclei in camel. Myoepithelial cells were observed between the acinar epithelial cells and its surrounding basement membrane (Figs 1G, I). The cytoplasm of the glandular epithelium showed PAS reactivity in sweat glands of both animals (Fig 1H, J).

### ***Immunohistochemical observations***

In both buffalo and camel, vimentin (VIM) immunostaining was demonstrated only in the dermis, meanwhile melanosome was demonstrated among the epidermal stratum basalis and hair follicles as summarized in Table (3). There were significant differences between expression of both VIM and melanosome in buffalo and camel, as the immunoreactivity of both was over-expressed in camel than buffalo (Figs 2, 3). The specificity of the immunostainings was confirmed by staining sections with non-immune serum as shown in Fig (4).

Concerning VIM immunoreaction, it was mainly expressed in the cytoplasm of the fibroblasts and endothe-

lia of blood capillaries in the papillary layer of the skin. Also, it was expressed in the cytoplasm of myoepithelial cells of the sweat glands (Figs 2A, B, C, D). Furthermore, fibroblasts around the sebaceous glands and hair follicles in the reticular layer of the skin showed detectable VIM expression (Figs 2E, F). Melanosome was distributed in the epidermis and hair follicles of the skin of both buffalo and camel. Melanosome was restricted to cells of the epidermal stratum basalis (Figs 3 A, B). Also, it was expressed in cells of the inner and outer root sheath of the hair follicles (Figs 3 C, D).

## Discussion

Skin of both Egyptian water buffalo and one-humped camel, like other mammals, was consisted of epidermis and dermis. The epidermis formed the outer most layer of the skin, which was its self-covered by a layer of keratin externally. It consists of the characteristic four strata; basalis, spinosum, granulosum, and corneum. Stratum basalis was characterized by the presence of melanin containing cells among their cells while a mild undulation appearance was the characteristic feature of the superficial horny layer and these results agreed with that mentioned by Quasem et al. (1992).

The dermis of both buffalo and camel was divided roughly into highly vas-

cular superficial papillary and deep reticular layers containing skin appendages; hair follicles, sebaceous and sweat glands along with fine pronounced collagen fibers. The previously mentioned result was similar to that of Quasem et al. (1992) and Atlee et al. (1997). The camel's dermis was characterized by the existence of extensive vascular plexuses than in buffalo suggesting more thermoregulation and water conservation functions of the camel's skin that was in agreement with Atlee et al. (1997).

However, the structure and distribution of skin appendages varied from other mammals. The present study detected coexistence of both simple and compound hair follicles in the skin of camel that was similar to the findings of Abdou et al. (2006) and Hekal (2014). On other side, only simple hair follicles were detected in the skin of buffalo that was in accordance with Jenkinsson (1965).

In both buffalo and camel, abundant sebaceous glands were observed in this study either in association with hair follicles or alone and that was in harmony with Quasem et al. (1992). However, sweat glands were present in all examined skin but they were located deeper to the dermis of camel than in buffalo, that suggests playing a role in evaporative cooling as thermoregulatory action, particularly



in hot climate, which is in agreement with Abdou et al. (2006). The structure of glandular sweat acini and their myoepithelium in camel was similar to that of other camelidae like llama (Atlee et al., 1997).

The morphometric study of the skin of buffalo and camel may assess them for the production of skin and leather industry. Also, thickening of the skin promotes protection of the animals against overheating in hot climate as stated by Shalimov and Katsy (1989) in the Angler cattle.

In the present study, the skin of buffalo was significantly ( $P < 0.05$ ) thicker than camel's skin where the thickness of the epidermis, papillary and reticular layers of the buffalo's skin was 178.06  $\mu\text{m}$ , 831.86  $\mu\text{m}$  and 3304.9  $\mu\text{m}$  respectively. The corresponding values in camel were 157.02  $\mu\text{m}$ , 621.37  $\mu\text{m}$ , and 3218.69  $\mu\text{m}$  respectively. These finding was similar to that of Hekal (2014) in camel. The significance of these parameters determines the surface pattern, and firmness and thickness of the skin as reported by Muralidharan and Ramesh (2005). Moreover, Kasem (2009) concluded that the dermis thickness which has deeper follicles produced strong skins with higher value of tensile strength.

In both buffalo and camel, VIM immunostaining was restricted mainly

to the dermis, while melanosome was restricted to the epidermal stratum basalis and root sheath of the hair follicles. The present study revealed localization of VIM in the fibroblasts and endothelia of the blood vessels, similar to that recorded also in the skin of the American buffalo and pig (Cho et al., 2006; Wollina et al, 1991) respectively. The wide spread distribution of VIM immunostaining in the epithelia, fibroblasts in papillary and reticular layers of the dermis, and endothelia of the blood vessels supports the importance of VIM as cytoskeleton filament protein as stated by Emam (2015).

Melanosome was localized only in melanocytes existing among the cells of stratum basalis of the epidermis. Such finding was in consistence with Kanitakis (2002) in skin of human. In the present study, we detected over-expression of VIM and melanosome in the skin of camel than of buffalo. More VIM content in the skin of camel may suggest higher filamentous proteins of camel's skin than buffalo in addition to, more pigmentation in the skin of camel, like other desert animals, may play a role in photo-protection (Yagil, 1985). Lerner and Fitzpatrick (1950) pointed out that ultra-violet radiation appears ways with melanin formation and thereby helps to increase pigmentation.

In conclusion, although there are similarity in the basic histological components of the skin of both Egyptian water buffalo and one-humped camel, certain histomorphometric and immunohistochemical characterizations were noticed. The present study indicated that buffalo's skin was thicker than camel, which put it up for the production of skin quality requirements of the leather industry. Furthermore, higher VIM content in camel's skin may suggest higher filamentous protein and tensile strength than in buffalo. It is also suggested that over-expression of melanosome in camel's skin than buffalo may play a role in photo-protection during long exposure to direct sunshine in the harsh hot climate.

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**Table (1):** Least square means ( $\pm$ SD) of skin layers thicknesses (epidermis, papillary and reticular layers).

Items	Buffalo ( $\mu$ m)	Camel ( $\mu$ m)
Epidermis thickness	178.06 $\pm$ 1.425*	157.02 $\pm$ 0.892
Papillary thickness	831.86 $\pm$ 11.926*	621.37 $\pm$ 16.159
Reticular thickness	3304.90 $\pm$ 71.952*	3218.69 $\pm$ 38.797

Means within the same raw differ significantly at \*.

**Table (2):** Least square means ( $\pm$ SD) of epidermal layers thicknesses (stratum basale, stratum spinosum, stratum granulosum and stratum corneum).

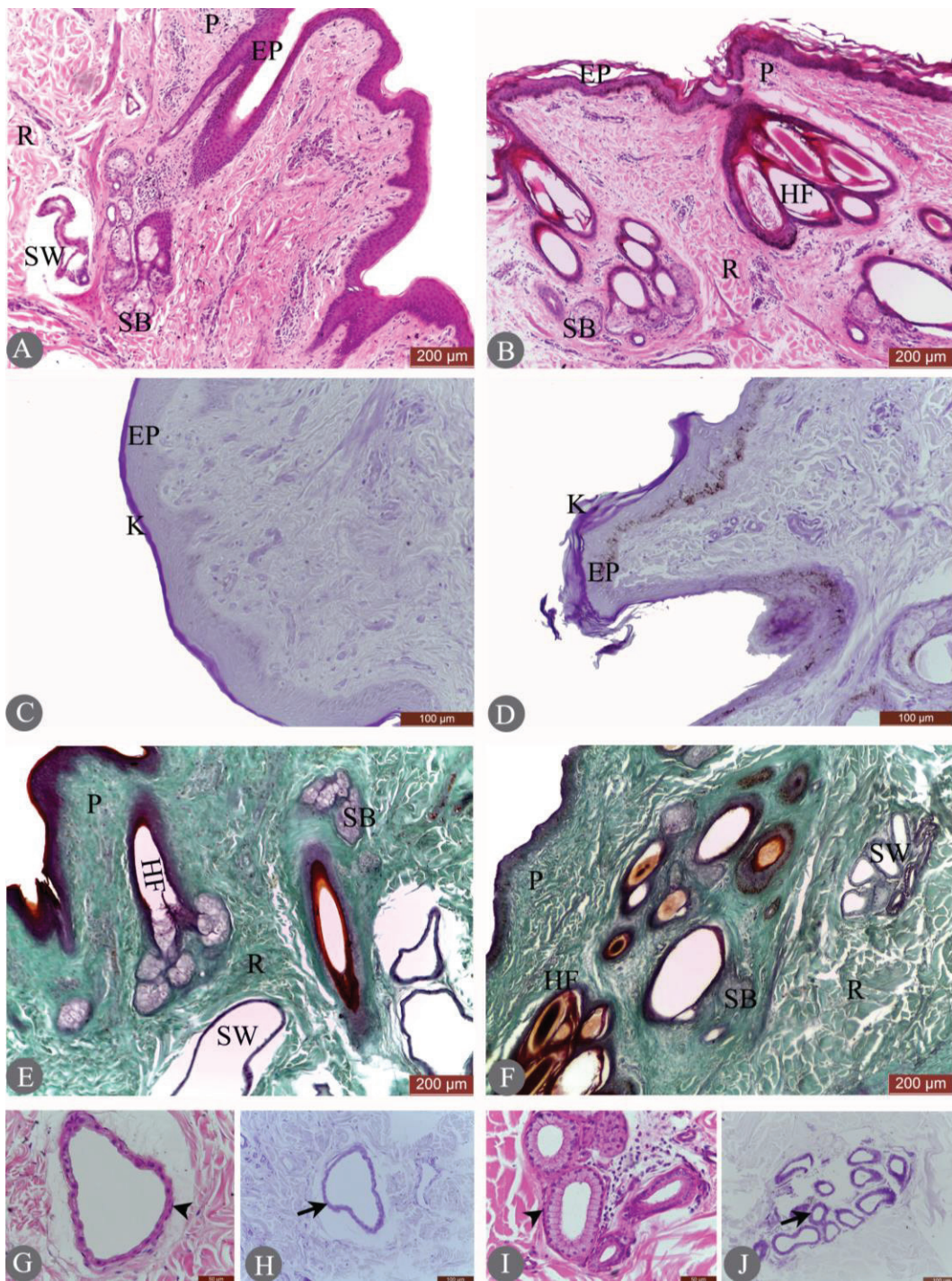
Items	Buffalo ( $\mu$ m)	Camel ( $\mu$ m)
Stratum basalis	25.07 $\pm$ 0.238	16.52 $\pm$ 0.097
Stratum spinosum	80.38 $\pm$ 1.016*	59.85 $\pm$ 0.994
Stratum granulosum	37.52 $\pm$ 0.491*	23.77 $\pm$ 0.617
Stratum corneum	36.84 $\pm$ 0.448	59.93 $\pm$ 0.806*

Means within the same raw differ significantly at \*.

**Table (3):** Summary of VIM and melanosome immunostainings in the skin of Egyptian water buffalo and one-humped camel.

	Buffalo		Camel	
	VIM	MS	VIM	MS
Stratum basalis	-	+	-	++
Root sheath of hair follicle	-	+	-	++
Fibroblast	++	-	+++	-
Endothelia	++	-	+++	-
Myoepithelium	++	-	+++	-

VIM, vimentin; MS, melanosome ; -, negative; +, slightly positive; ++, moderately positive; +++, strongly positive.



**Fig (1): Photomicrograph of the skin of buffalo (A, C, E, G, H) and camel (B, D, F, I, J).**

**A & B:** showing general structure of the skin. EP, epidermis; P, papillary layer; R, reticular layer; SW, sweat gland; SB, sebaceous gland; HF, hair follicle. H&E stain.

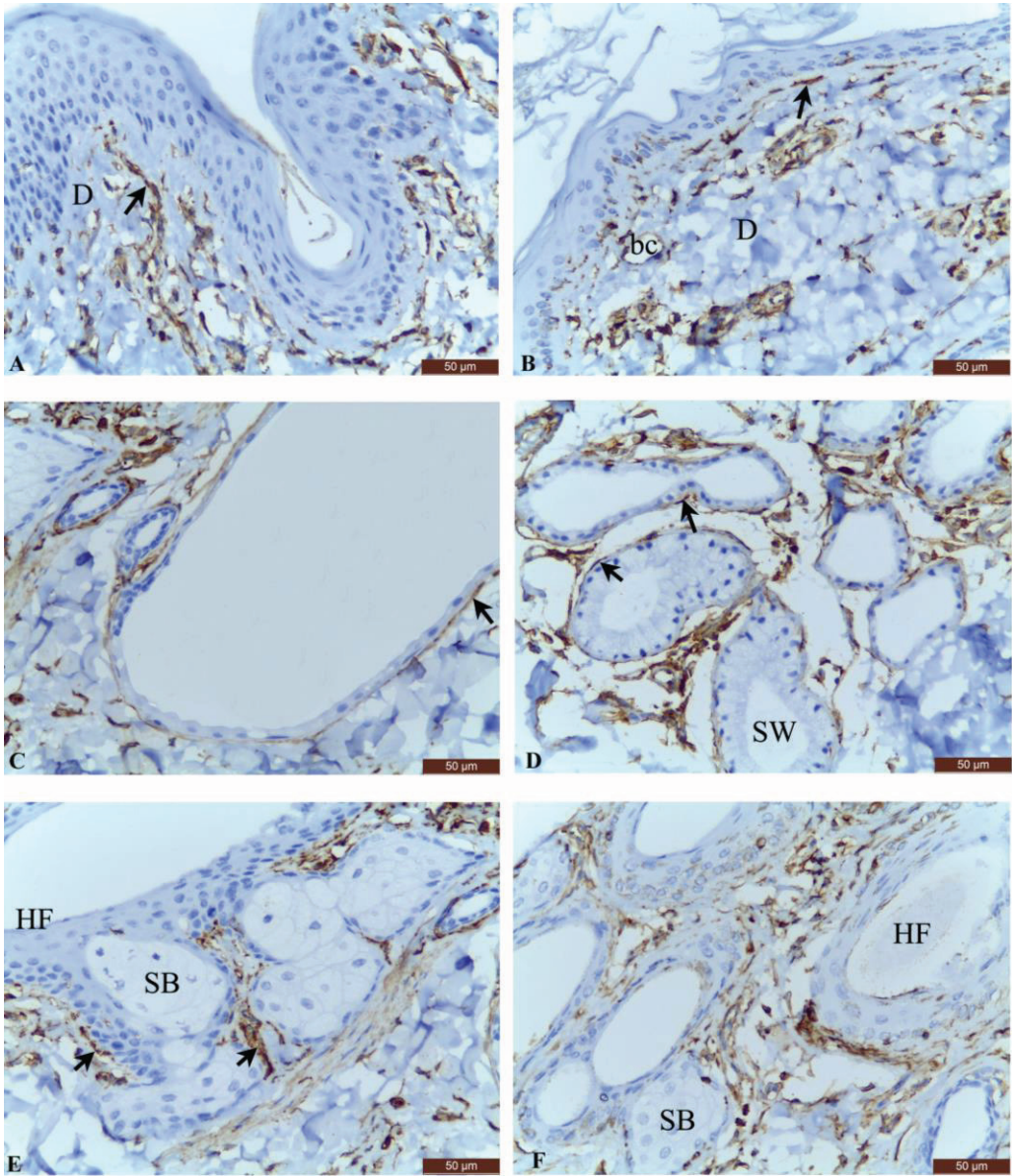
**C & D:** showing PAS positive keratin layer of the skin. K, keratin; EP, epidermis. PAS technique.

**E & F:** showing component of the dermis. P, papillary layer; R, reticular layer; SW, sweat gland; SB, sebaceous gland; HF, hair follicle. Crossman's trichrome stain.

**G - J:** showing the structure of sweat glands. Arrowhead, myoepithelium; arrow, positive PAS glandular cells. PAS technique.

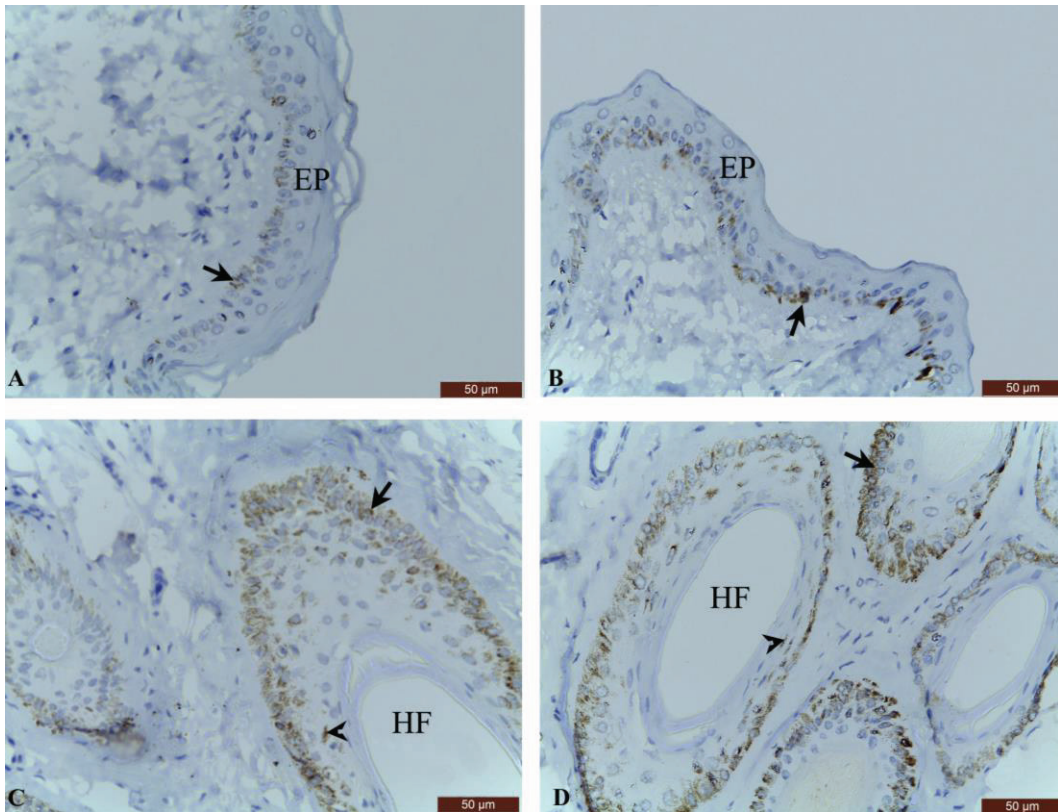
Scale bars (A, B, E, F) = 200  $\mu$ m; (C, D, H, J) = 100  $\mu$ m; (G, I) = 50  $\mu$ m.





**Fig (2): Photomicrograph of vimentin immunostaining in the skin of buffalo (A, C, E) and camel (B, D, F). A & B:** showing VIM immunostaining in fibroblasts (arrow) and endothelia of blood capillaries (bc) in dermis (D) of skin. **C & D:** showing VIM immunostaining in myoepithelium (arrows) surrounding the sweat glands (SW). **E & F:** showing VIM immunostaining in fibroblasts (arrow) around sebaceous glands (SB) and hair follicles (HF). Scale bars = 50 µm.



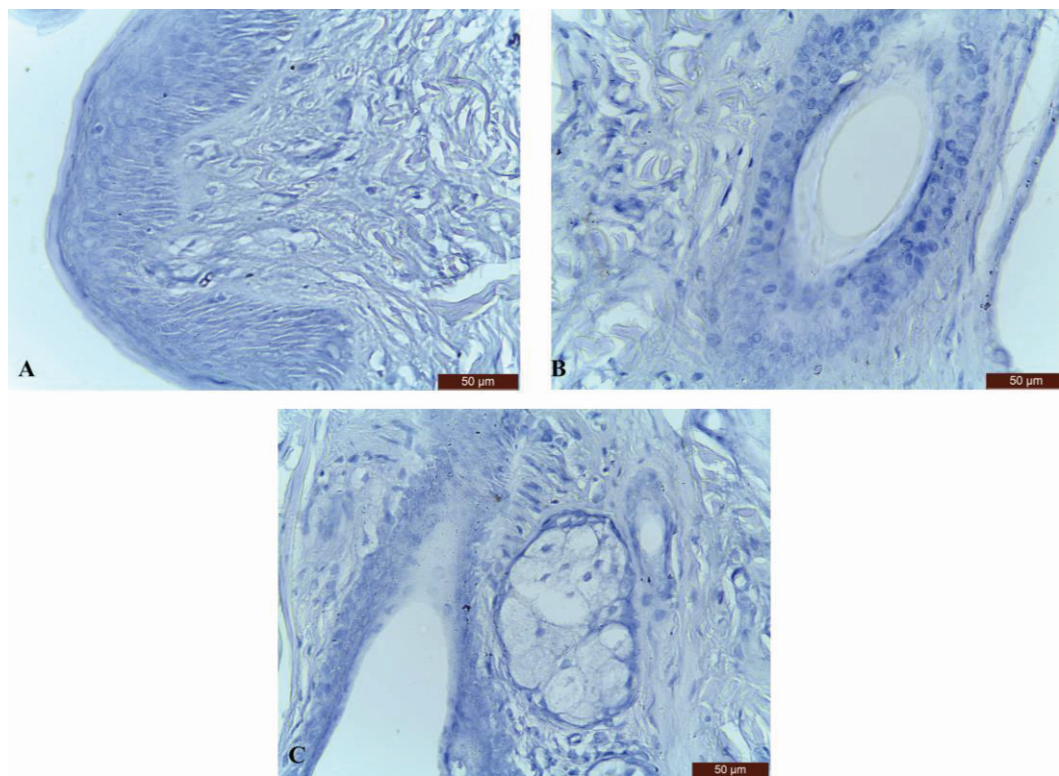


**Fig (3): Photomicrograph of Melanosome immunostaining in the skin of buffalo (A&C) and camel (B&D).**

**A & B:** showing Melanosome immunostaining in melanocytes (arrow) among the cells of stratum basalis of the epidermis (EP).

**C & D:** showing Melanosome immunostaining in cells of the inner and outer root sheath (arrows) of hair follicles (HF).

Scale bars = 50 µm.



**Fig (4): Photomicrograph showing negative control sections of the skin of buffalo (A) and camel (B&C) using non-immune serum. Scale bars = 50 µm.**