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Histological characterization of the skin of the *Paraechinus hypomelas*, Brandt, 1836 (Erinaceidae: Eulipotyphla)

Running head: Histology of the skin of *Paraechinus hypomelas*

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Abstract

The current study represents the first description of the histological characterizations of the normal skin of Brandt's hedgehog(*paraechinushypomelas)*. Skin samples were collected from abdomen, back, nostril and cloacal regions. The skin consisted of three layers including epidermis, dermis and hypodermis. The epidermis was covered by a layer of keratinized squamous epithelium mainly in the back region, but nearly was present with a little amount or may was absent in other regions. Histologically, the total thickness of skin was maximum on the back and minimum on the cloacal regions. The epidermis consisted of four layers and stratum

lucidum was absent in all regions. Beneath the epidermis, the dermis layer was constituted of dense connective tissue in which the hair follicles, sweat glands, sebaceous glands, arrector pilli muscles and blood vessels were present. The sweat and sebaceous glands were more populated in the nostril region. The hair follicles were located in the epidermal and dermal regions. Vibrissae wereonly in the nostrils region and characterized from other hairs by their large and well innervated hair follicle which was surrounded by the blood sinus.
Key words: hedgehog, histology, skin, sweat gland, *Paraechinus hypomelas*

INTRODUCTION

The hedgehog is described as a spiny mammal species.Generally, there are 17 species of the hedgehog which are classified in five genera and distributed in certain parts of Asia, Africa, and Europe. The Brandt's hedgehog (*paraechinus hypomelas*) species belongs to the *Eulipotyphla* order, *Erinaceidae* family, *Erinaceinae* subfamily, and *Paraechinus* genus [21]. The Brandt's hedgehog is mainly nocturnal species and lives in the desert regions of the Middle East and Middle Asia [21]. The animal integument givesthe mechanical protection of the underlying tissue and acts as a barrier against external infection and the different environmental conditions [10]. In addition to thebiological and immunological values, the skin has an important economic importance in the industry as a raw material which introduce in many important industry [13]. The structure of the skin can be influencedby several factors including living environment (temperature, humidity, etc.) and nutritional habits. Therefore, thickness orstrength (collagen content) of skin layers is species-specific. In addition, the skin of different regions canvary regarding to the thickness of layers, type of glands, and presence or density of hair follicles [6]. For instance, Kietzmann et al.revealed that rat's tail has histologically different epidermis in comparison to other parts of the body [7]. Furthermore, gender and age in a given species can influence the skin histology. It has been shown that, in rat the thickness of dermis increases with age, so that it reaches up to 5 times in one year [19]. There are some morphological descriptions of the skin of other mammals' species such as ferret, camel,and cat [1, 9, 23]. However, there is a scanty available data about the normal properties of the skin of hedgehog especially, the Brandt's hedgehog (*Paraechinus hypomelas*) species. So, the current study was conducted to explain the histological structure of the skin of Brandt's hedgehog captured from Kermanshah city. Finally, the obtained results were compared with those reported in other mammalian species.

MATERIALS AND METHODS

Samples collection

Five male animals were included in the present study. The dead animals were found around the rural regions of Kermanshah city and transferred to dissection room of Faculty of Veterinary Medicine. The exact time of death was unclear, however, based on the appearance of the viscera, it seemed less than two hours past from their death.The present study performed according to the guideline of committee on Animal Welfare and Ethics of Razi University.

Microscopic examination

Small specimens (1 cm^3×1 cm^3) of the skin from four different regions of the body including nostril, back, abdomen and cloacae were sampled. The skin samples were fixed in 10% neutral buffered formaldehyde for 10 days. For histological observation, skin samples were routinely processed and embedded in paraffin. The histological sections with 5–7 µm thicknesswere prepared usingLeica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. Paraffin sections were used for conventional staining (Hematoxylin and Eosin), in addition to Masson trichrome and Periodic Acid Schiff (PAS) staining methods. Finally, the sections were analyzed and epidermal (corneal and cellular), dermal and hypodermal thicknesswere measured using a light microscope(NikonYS100,Japan) equipped with a KEcamera (KEcam Technologies, Lekki Lagos, Nigeria) and Top view software (Version 3.7). The images were processed using Adobe Photoshop CC (Adobe system, San Jose, CA, USA).The density of sebaceous and sweat glands were calculated in different regions of the Brandt's hedgehog using a lattice line graticule (5 × 5).

Data analysis

Data were analyzed using the one-way ANOVA in SPSS/PC software (Version 23) followed by Tukey's *post-hoc* test.All values were expressed as mean ± standard error.

RESULTS

Histometrical study

The thickness of skin layers in four different examined regions were described in the Table 1.The mean values of total dermal thickness in abdomen, back, cloak and nostril regions were 440.9±45.8 μm, 2041.9±190.9 μm, 468.9±61.8 μm and 413.6±45.9 μm, respectively. The epidermis of the back region had thickest keratinized layer (45.6±10.2 μm), while the thinnest keratinized layer was seen in the abdominal region (17.6±6.9 μm). The keratinized layer was absent in the cloacal region. The thickest and thinnest cellular part of the epidermis were measured in the nostril and cloacal regions, respectively (140.3±20.6 and 17.4±8.7 μm). The highest value for the hypodermal thickness was1150.9±170.9 μm in the back and the lowest value was 125.9±25.4 μm in the nostril region (Table 1).

The density of the sebaceous and sweat glands in the nostril region (8.61±0.95 and 9.51±1.34 unit/mm^2, respectively) was significantly higher than other examined regions. The difference between back, abdominal and cloacal regions was not statistically significant (Table 2).

Histological study

The micromorphological structure of the Brandt's hedgehog (*paraechinushypomelas)*skin consisted of three layers namely epidermis (external layer), dermis (middle layer) and hypodermis (internal layer) (Figs.1, 2A,B, 3Band 5). The epidermis was covered externally by a thick layer of keratinized material in the back region (Fig. 3B), but a little amount of cornification was present over the abdominalepidermis (Figs. 1 and 2A,B).

The cellular part of the epidermis consisted of four layers especially in the nostril region and arranged from outside to inside as the following; stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Figs.5A, 6B and 7B). These layers were not distinguishable clearly in the abdominal, back, and cloacal regions (Figs.1, 2A,B, 3B and 8). The excretory ducts of sweat glands were seen in cellular epidermis of the nostril region (Figs.5A, 6B and 7B). Thedermis constituted of dense irregular connective tissue especially in the back and nostril regions with highly compact collagen bundles (Figs.3B and 5). But it was more loose in the abdominal and cloacal regions (Figs.1 and 8). There was many important structures including sweat gland, hair follicle, and nerve ending in all examined region, sebaceous gland in all examined regions except in the cloacal region, spine follicles in the back region (Fig. 4A-D), and vibrissae (tactile hair) in the nostril region (Fig. 6A). The interdigitation between the dermis and epidermis as dermal papillae and epidermal peg was more prominent in the nostril region (Figs.5, 6 and 7).

The hypodermis consisted of loose connective tissue and adipose tissue (Figs.1, 2A,B, 3A and 8). In the back region, this layer contained a huge amount of striated muscle fibers which surrounded spine follicles (Figs.3A and 4B, D).

DISCUSSION

The present work attempted to describe histological properties of the skin in Brandt's hedgehog (*Paraechinus hypomelas*). Previous studies have shown that the thickness of different layers of skin (dermis and epidermis) and its structures depends on various factors such as age, gender, ethnicity, living environment, nutrition, etc [12]. Micromorphologically, the skin of the Brandt's hedgehog consisted of three layers namely epidermis, dermis and hypodermis as described in many animal species [20]. While, in the Bakerwali goat,and cattlethe skin has been described that consisted of two layers; epidermis and dermis [14, 17].

Several studies have shown that the thickness of epidermis depends on a variety of factors, including the position of skin in the body, age, gender, pigmentation, and the amount of blood supply. One study on human skin showed that the thickest epidermal layer (81.5 µm) was in the buttock area and the thinnest (56.6 µm) in the upper back area of the upper limb. The highest thickness of stratum corneumof the epidermis was related to the upper dorsal area of the body (18.3 µm) and the lowest thickness (11 µm) was in the shoulder.The corneum layer was less related to age and gender changes than the cell layer [15]. The thickest skin with thickest corneum layer was observed in the back area of the hedgehog, while the cloacal area had the thinnestskin. This difference may be due to the water retention, presence of spines and absorption of light by the back region. Theerawatanasirikul et al.examined the thickness of epidermis in different parts of the body according to the age and gender of the dog. They found that the epidermis thickness was 35 mm in male dogs and 36.10 mm in female ones. Thickness of the epidermis was 33.2 mm in dogs less than 3 years old and 37 mm in dogs older than 7 years. This difference may be due to the protective role of the skin to prevent water withdrawal [18].

Corcuff et al.calculated the thickness of human epidermis using a confocal microscope. The thickness of granulosum layer was 12-15, and the stratum spinosum was reported to be 9-12 µm. In addition, the thickness of stratum basale was 6-8µm, and overall thickness of epidermis was 36 µm [3]. Sumena et al.reported the thickness of skin in the muzzle, neck, abdomen, and ankles of Yorkshire white pig to be 4, 3.7, 3.68, and 4.15 mm, respectively. The hypoderm was not included in thickness [16]. In studying the effect of age on human epidermalchanges, it has been revealed that the thickness of the epidermis was reduced from 49 µm in women and 63 µm in men aged 20 to 30 years, to 33 µm in women and 33 µm in men aged 70to 80years. However, the thickness of dermis was increased from 212 µm in women, and 256 µm in men aged 20-30 to 251µm in women and 257 µm in men aged 70to 80 years [2]. The epidermis of the Brandt's hedgehog consisted mainly of four layers which arranged from outside to inside as the following; Stratum corneum, Stratum granulosum, Stratum spinosum, and Stratum basale as described by some authors[14, 20].Furthermore, at the junction between the epidermis and dermis, epidermal pegs were interdigitated with corresponding dermal papillae especially in the nostril region. These results noted also by Razvi et al. [14].

Thickness of various layers of skin depend on the animals breed. In the Mertolenga race of Portuguese native cattle, the thickness of corneum layer of skin is 2.6µm, while in the Friesian race it reaches 8 µm. Thickness of the cell layers Friesian race is 38 µm, but it reaches 49 µm in the Limousin race as well [11]. Dorsal dermis contains mainly thorns that cover the surface of the skin. These thorns are loose in the lower layers of the dermis. This feature improves the movement of the animal in small areas. The hypodermic layer is very thick in the back area and extends even to the epidermal folds. Below the hypodermis, there is a thick muscular layer called panniculus carnosus. ZAki found that thickness of epidermis reduced by age in back, earlobe, and forefoot of albino rat aged 1, 2 and 27 months. The thickness of epidermis in these areas at 6 months of age, as the puberty age, was reported to be 12, 9.4, 13.3, and 48 µm, respectively.In addition, the thickness of pilosebaceus was 20, 21.9, 30.6 and 11.3 µm at this age [22].

The thickness of the stratum corneum was reported 93, 14 and 16 µm, respectivelyin cow, human and rat lip. Thickness of stratum spinosum was 527, 44 and 91 µm, stratum basale 54, 7 and 11 μm, and the granulosum layer was reported only 7 μm in rats. These differences may be caused by the role of lips in feeding, preserving water and reducing body temperature in different animals[8]. The highest amount of sebaceous glands was seen in the muzzle area(nostril) of the Brandt's hedgehog, while there was no sebaceous gland in the cloak region. Most of the sweat glandular acini were found in the muzzle section and the least sweat glands wereseen in the abdominal and cloacal regions.In native cattle of Bangladesh, the highest number of sebaceous glands was reported in the foot area (2.08 mm^2) and the lowest number in the shoulder area (0.46 mm^2). Furthermore, the number of glands in females were significantly more than males [4]. The vibrissae hairs named as whiskers and described as specific tactile sense hair found in most mammalian species except humans and were located exclusively in the nostril region of the Brandt's hedgehog. They were also observed in ferret**,** dog, and rat [5, 9, 20].

The presence of elastic fibers in the back of the Brandt's hedgehog was noticeable in comparison to other parts, while in the cloacal region, these strands were observed rarely. Most blood vessels and nerves were seen in the muzzle area which can be served for sense of touch and concentration of mucosal secretions. There were a lot of blood vessels around the follicles of the spines in the back area. Pacinian corpuscles contribute to the sense of tension and compression of the skin in the reticular dermis of the abdomen. Skeletal muscles of skin were placed in the vicinity of the spines in the back region and contributed to their movement. The hair follicles were numerous in the muzzle area and extended to the reticular portion of dermis. They used in breathing, sense of smell and tactile sensation of the Brandt's hedgehog. The mucosal glands and their pores were seen in the muzzle and play a role in keeping this area wet. A layer of fat was observed in the abdominal andcloacalregions. This layer was not well developed in the back and the muzzle. Because of hibernation in some animals, the thickness of skin in different areas of some animals such as the hedgehog, and distribution of sebaceous and sweat glands varied from other animals, because there is a need for water retention and avoidance of the energy loss through heat. Sebaceous glands are dependent on the animal's living environment, gender, and season. The foods affecting the level of sex hormones which play a role in the number and distribution of these glands. Sebaceous glands, which are altered by age in animals, are 8.6 μm in lactation, 4.2 μm in puberty and 3.2 μm in old ages [23].

Overall, the present findings show that in Brandt's hedgehog (*paraechinushypomelas*)theback and cloacal regions have thickest and thinnest skin respectively as compared to the nostril and abdominal regions. In addition, Sebaceous and sweat glands were mainly populated in the nostril region of the body.

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Body regions	Epidermal thickness		Dermal thickness	Hypoderm	Total thickness
	Corneal	Cellular			
Abdomen	17.6±6.9[a]	30.9±10.9[a]	440.9±45.8[a]	352.8±60.6[a]	560.06± 68.44[a]
Back	45.6±10.2[b]	20.6±7.9[ab]	2041.9±190.9[b]	1150.9±170.9[b]	2226.60±140.60[b]
Cloak	-	17.4±8.7[b]	468.9±61.8[a]	249.9±50.8[a]	520.32±45.22[a]
Nostril	30.8 ±8.1[c]	140.3±20.6[c]	413.6±45.9[a]	125.9±25.4[c]	620.14±14.26[a]

Table 1. Thicknesses (μm) of skin layers in Brandt's hedgehog (n=5). Values are expressed as mean ± standard error.

Different superscripts in the same columnindicate significant difference between regions (P < 0.05).

Table 2. The density of the sebaceous and sweat gland in the Brandt's hedgehog skin (n = 5). Values are expressed as mean ± standard error.

Body regions	Sebaceous gland/ mm^2	Sweat gland /mm^2
Abdomen	3.41±0.68[a]	3.32±0.24[a]
Back	3.52±0.32[a]	4.23±0.81[a]

Cloak	-	3.25±0.15[a]
Nostril	8.61±0.95[b]	9.51±1.34[b]

Different superscripts in the same columnindicate significant difference between regions ($P < 0.05$).

Figure legends:

Figure 1. Light photomicrograph of the normal skin of the abdomen region in the Brandt's hedgehog. Stratum corneum (SC), Stratum spinosum (SS), Stratum basale (Stb), Epidermis (Ep), Dermis (D), Hypodermis (Hy), Sebaceous gland (SBG), Hair shaft (Hs), Adipose tissue (Dt), Pacinian corpuscle (PCO), External root sheath (1), Internal root sheath (2), Hair cortex (3). H/E, Bar = 100 µm, ×40.

Figure2. Lightphotomicrograph of the normal skin of the abdomen region in the Brandt's hedgehog.H&Estaining **(A)** and Masson's trichromestaining **(B)**. Epidermis (Ep), Dermis (D), Sebaceous gland (SBG), Adipose tissue (Dt), Hair shaft (Hs), External root sheath (1), Internal root sheath (2), Hair cortex (3). Bar = 100 µm,×40.

Figure 3. Light photomicrograph of the normal skin of the back region in the Brandt's hedgehogshows mass ofSkeletal muscle (SKM), Adipose tissue (Dt) **(A)**, and shows thick keratinized layer of epidermis (Ke), and Dermis (D) (**B)**. Masson's trichrome staining, Bar=100 µm, ×100.

Figure 4. Light photomicrograph of the normal skin of the backregion in the Brandt's hedgehog. Masson's trichrome staining **(A** and **B)**, and H&E staining **(C** and **D)**. Bright pink basement membrane (bm) at the dermal-epidermal junction around the spine follicle (SF) in cross section, Spine root (SR), Skeletal muscle (SKM), Arrector pilli muscle (Am),Upper portion of hair shaft (infundibulum) (U). Bar =50 µm, ×100.

Figure 5. Light photomicrograph of the normal skin of the nostril region in the Brandt's hedgehog. without hair follicles **(A)** and with hair follicles **(B)**. Keratinized material (Ke), Stratum corneum (SC), Stratum spinosum (SS), Stratum granulosum (Sg), Stratum basale (StB), Epidermis (Ep), Dermis (D), Basement membrane (Bm), Epidermal peg (Edp), Dermal papillae (Dp), Hair follicle (HF), Sweat gland (SG), excretory ducts of the sweat glands (white arrowheads). Masson's trichrome staining,Bar = 100 μm, ×100.

Figure 6. Light photomicrographs of the normal skin adjacent to the nostril with thin epithelium containing vibrissae hair **(A)** and with thick epithelium **(B)** in the Brandt's hedgehog.Keratinized material (Ke), Epithelium (Ep), Dermis (D), Stratum corneum (SC), Stratum spinosum (SS), Stratum granulosum (Sg), Stratum basale (StB), Epidermal peg (Edp), Dermal papillae (Dp), Vibrissae hair follicle (VH), Hair follicle (HF), Hair shaft (Hs), Sweat gland (SG), Sebaceous gland (SBG), Skeletal muscle (SKM), excretory ducts of the sweat glands (white arrowheads), Capsule (CP), Sinus (S), inner root sheath (1), outer root sheath (2), Glassy membrane (Gm). (H/E, Bar = 200 μm, ×100.

Figure 7. Light photomicrograph of the normal skin of the nostril region in the Brandt's hedgehog **(A)**. Red rectangle shown in higher magnification**(B)**. Keratinized material (Ke),Stratum corneum (SC), Stratum spinosum (SS), Stratum granulosum, Stratum basale (StB), Dermis (D), Hair follicle (HF), Hair shaft (HS), Capsule (CP), Epidermal peg (Edp), Dermal papillae (dp), Sebaceous gland (SGB), Inner root sheath (In), Outer root sheath (Or), excretory ducts of the sweat glands (white arrowheads). (Masson's trichrome staining, Scale bar 200 μm)The red rectangle shown in B at higher magnification. Bar = 100 μm, ×100.

Figure 8. Lightphotomicrograph of the normal skin of the cloacal region in the Brandt's hedgehog with Masson's trichrome staining **(A)** and H&E staining **(B)**. Dermis (D), Skeletal muscle (SKM) in hypodermis, Blue headarrows refer to cross section of nerve, Bar = 100 μm, ×40.

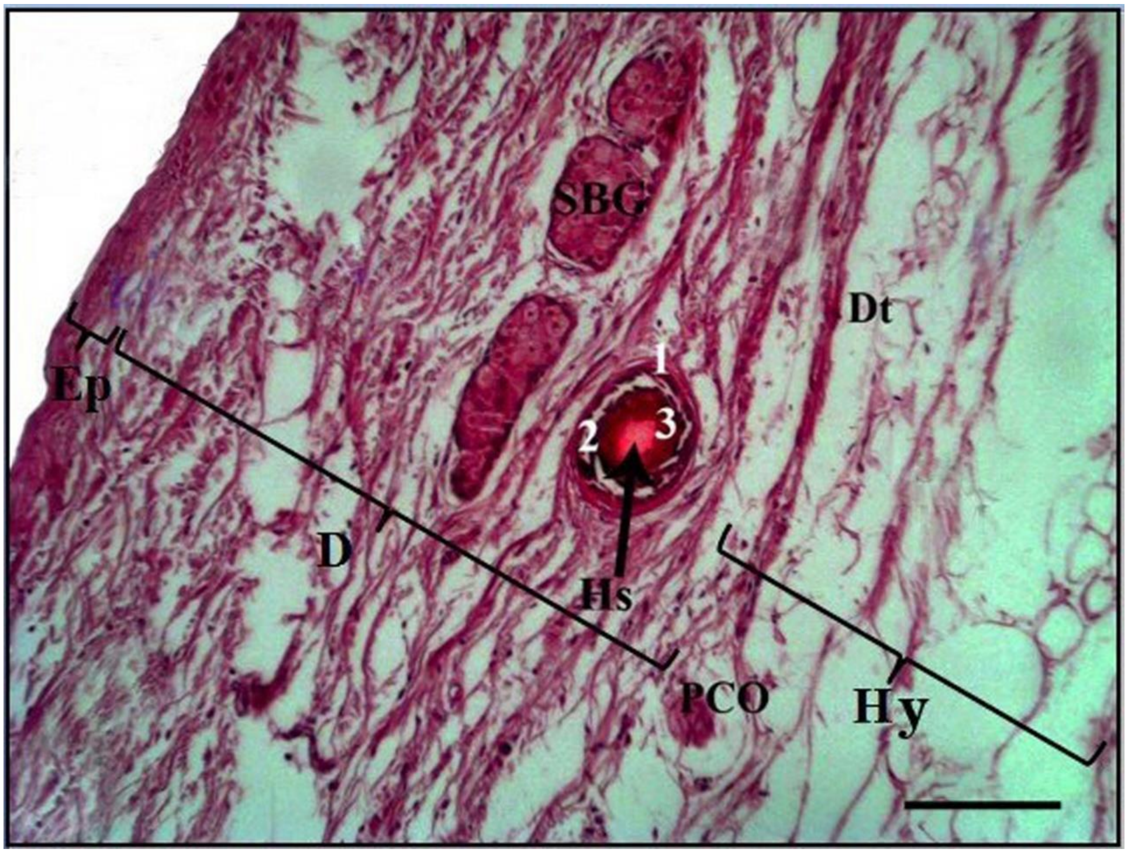


Fig. 1

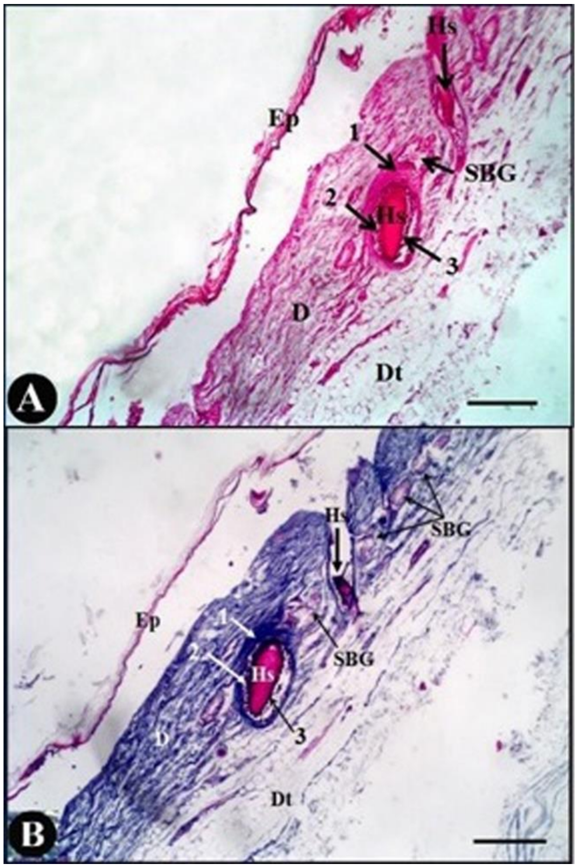
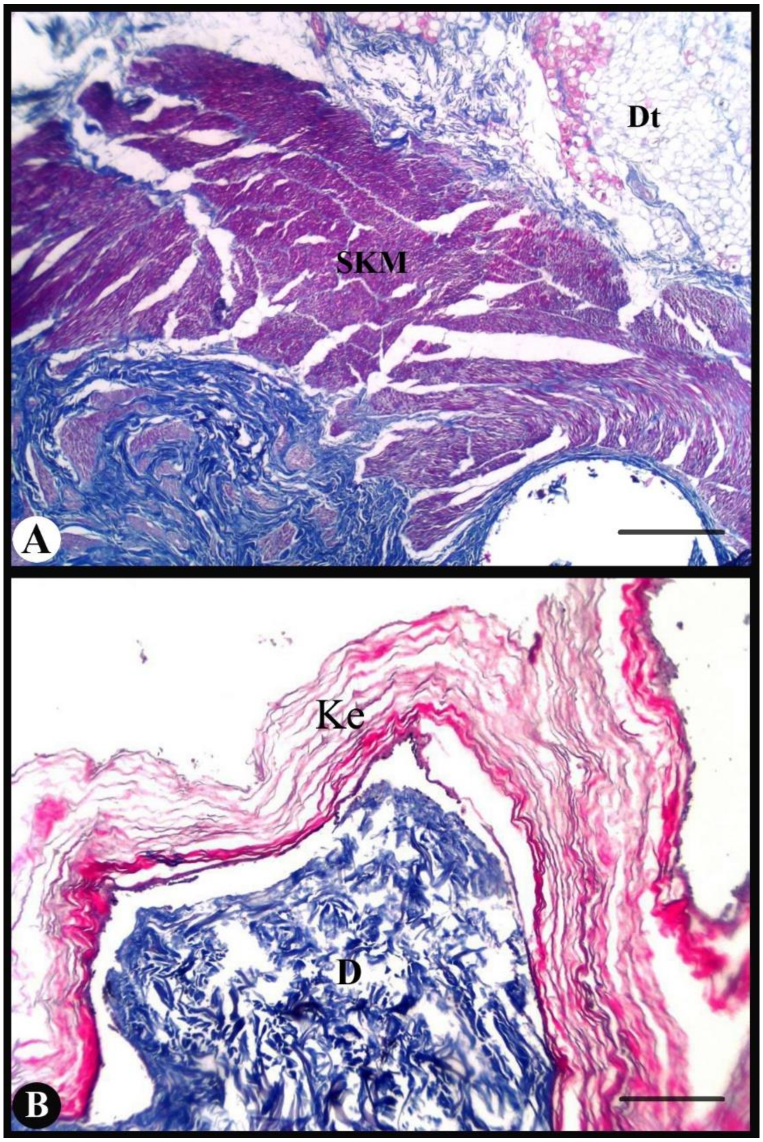


Fig. 2



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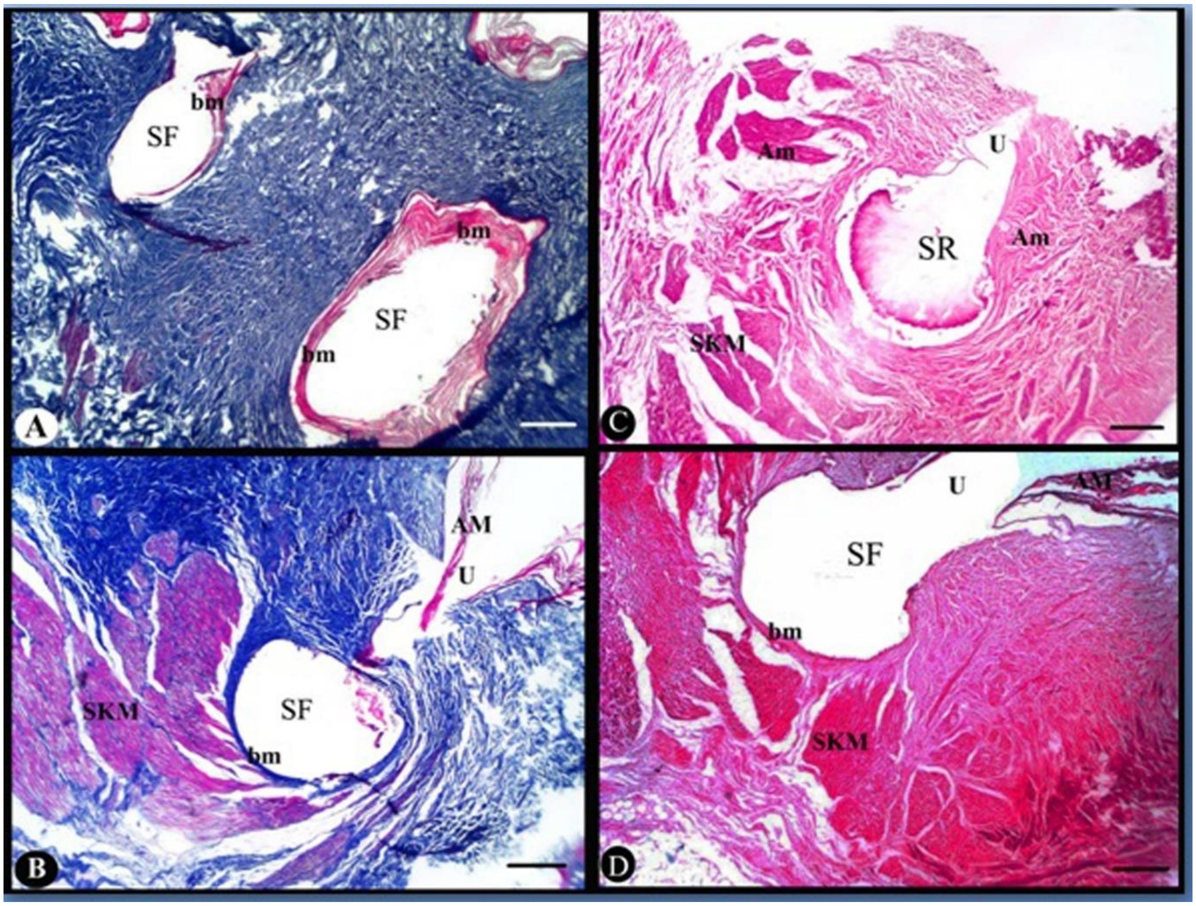

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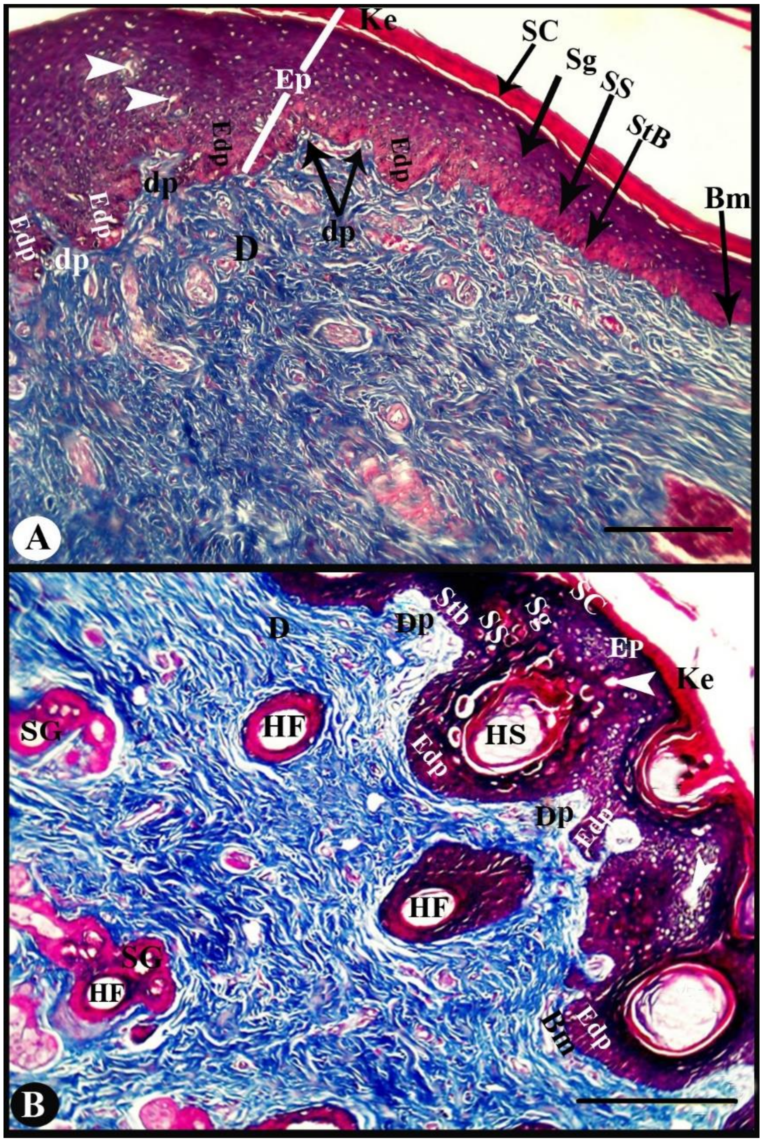

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Fig. 5

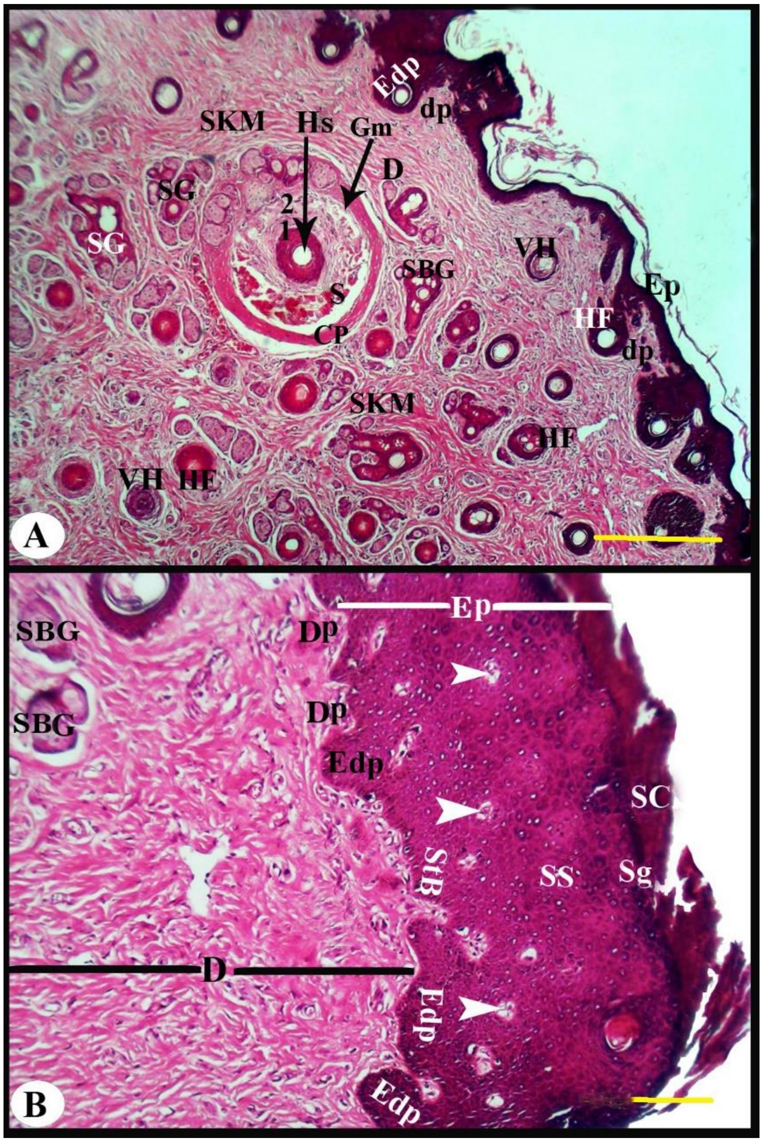

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Fig. 6

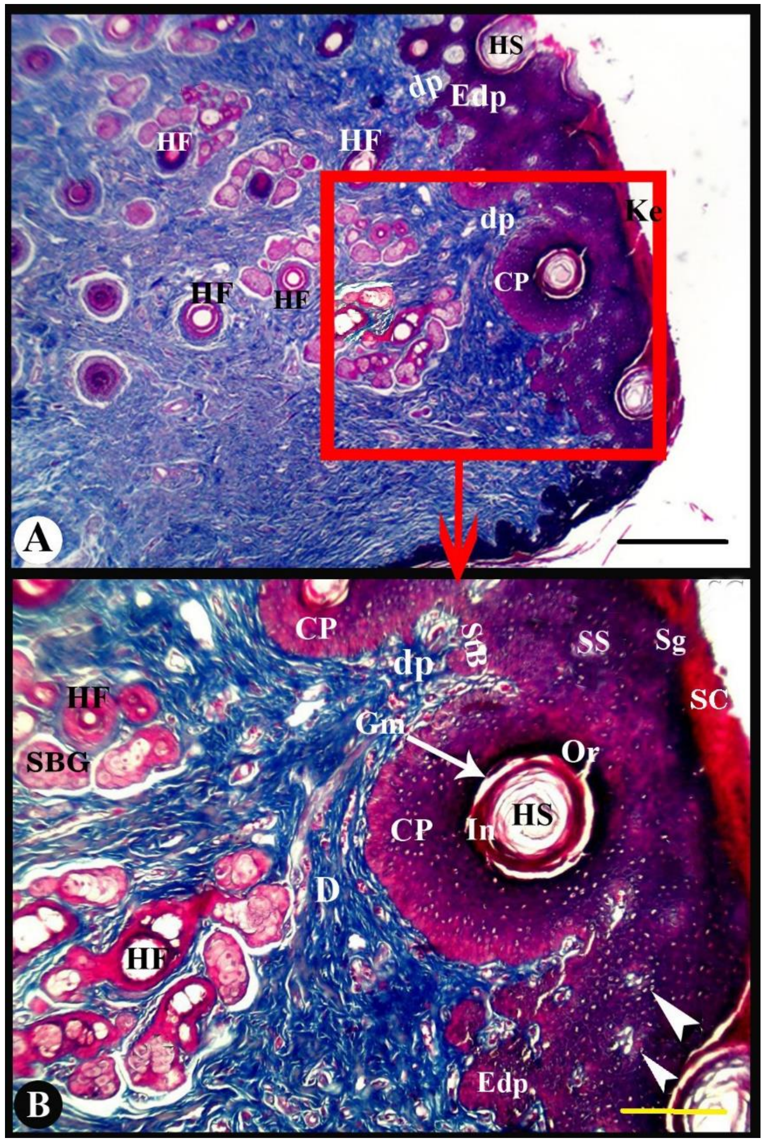
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Fig. 7

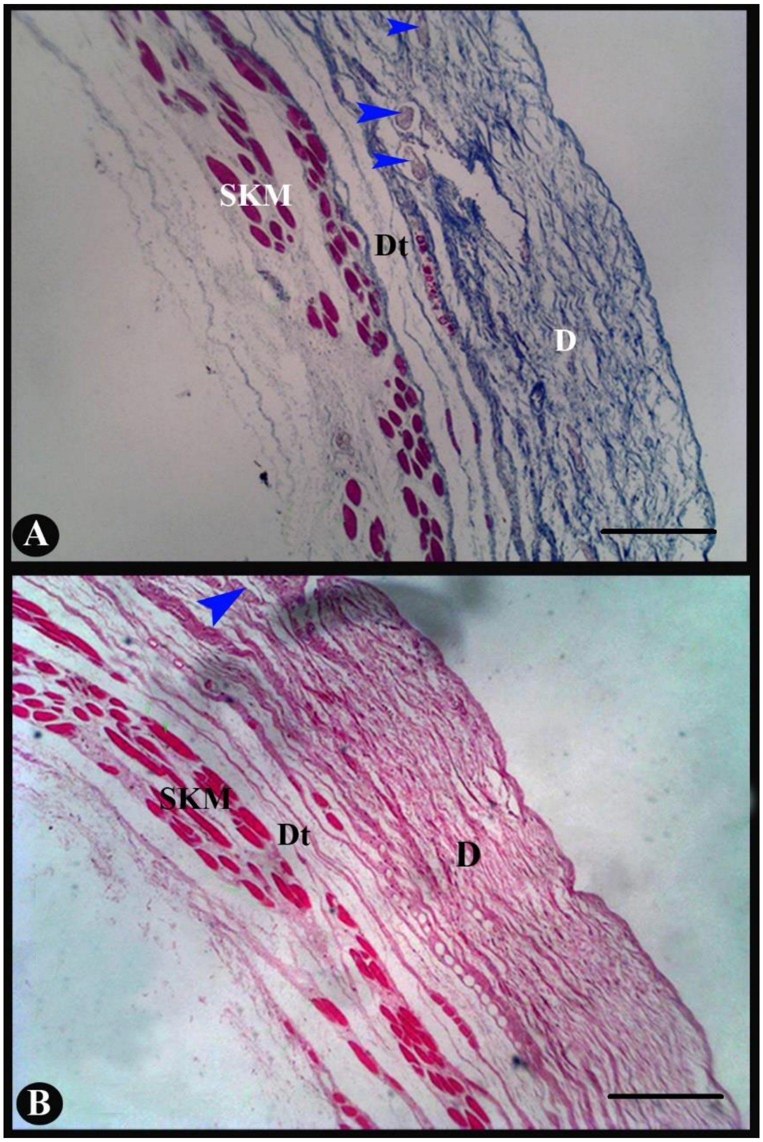

Fig. 8