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Research Article

EARLY EFFECTS OF TATTOOING ON MOUSE SKIN MORPHOLOGY AND MAIN DIFFERENTIAL FEATURES WITH NORMAL SKIN

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ABSTRACT

The authors performed a histomorphological analysis of 40 mice dividing into two groups:1-non-tattoed (20 mice) and 2- tattooed in the base of their tale skin (20 mice) in the period from 2022-2023. During the research early effects (from tattooing to day 14) of tattoing in the tail mouse skin was observed. The results of the study showed that almost all (99%) observed tattoed skin had a black pigmentation. (%) acute inflammation in epidermis, (%) epidermal necrosis and (%) dermal hemorrhage during 1-7 days.A week later regenerative epidermal hyperplasia (%),chronic active dermal inflammation (%) and dermal necrosis (%) were recorded.

KEYWORDS

Tattooing, black tattoo, exogenous pigment, histomorphological structures, formaline, epidermal necrosis, dermal hemorrahage, regernerative epidermal hyperplasia, reactive lymphoid dermis, vacuolar basal epidermic degeneration.

INTRODUCTION

Tattoo is a form of body modification done by inserting indelible ink into the dermis to change its pigmentation. Tattoos are done for social, cultural, and religious purposes. It has been in existence since the

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18th century and was associated with sailors, lower class individuals, and criminals. However, since the late 20th century, tattooing has undergone a redefinition and shifted to an acceptable form of expression all over the world, including Uzbekistan, cutting across almost all age groups and socioeconomic class. [1,3,9,23]. The dermatological complications associated with tattoos can occur either during inking or attempts at removal. Most times, tattoos are obtained through unsafe means by unauthorized personnel, and this is associated with numerous health risks. Of particular importance to the dermatologists are the hypersensitivity reactions, granulomatous skin disease, and formation of both keloid and hypertrophic scars. The trend of tattooing has become a widely accepted form of social expression all over the world and is gradually gaining ground in Uzbekistan too. Unfortunately, tattoos cause a broad range of clinical problems [2,3,4.5,7,22].

Mild complaints,specially sensitivity to sun, are very common and seen in 1/5 of cases. Medical complications are dominated by allergy to tattoo pigment haptens or haptens generated in the skin,especially in red tattoos but also in blue and green tattoos. Symptoms are major and can be compared to cumbersome pruritic skin diseases. Tattoo allergies and local reactions show distinct clinical manifestations, with plaque-like, excessive hyperkeratotic, ulcero-necrotic, lymphopathic, neuro-sensory,and scar patterns. Reactions in black tattoos are papulo-nodular and non-allergic and associated with the agglomeration of nanoparticulate carbon black. Tattoo complications include effects on general health conditionsand complications in the psycho-social sphere. Tattoo infections with bacteria, especially staphylococci, which maybe resistant to multiple antibiotics, may be prominentand may progress into life-threatening sepsis. Contaminated tattoo ink is an open-window risk vector that can lead to epidemic tattoo infections across national borders due to contaminated bulk production. Hepatitis B and C and human immunodeficiency virus (HIV) transferred by tattooing remain a significant risk needing active prevention. It is noteworthy that cancer arising in tattoos, in regional lymph nodes, and in other organs due to tattoo pigments and ingredients has not been detected or noted as a significant clinical problem hitherto, despite millions of people being tattooed for decennia [6,8,10,11,15]. Clinical observation and epidemiology disagree with register data, which indicate an increased risk of cancer due to chemical carcinogens present in some inks.

Patients frequently present to the dermatologists and physicians for solutions to the complications which are above mentioned. It is important to proffer solutions and educate patients on the various health risks associated with tattooing [13,14,16].

Objects of research: In order to reduce the harmful effects caused by tattooing, to study its early effects on skin morphology in mouse tail skin. At the same time, comparing the morphological aspects of normal and tattooed mouse tail skin.

MATERIALS AND METHODS

The biomaterial for histological examination was fresh pieces of skin cut from two different sources: normal and tattooed tail skin of mice. The size of the pieces did not exceed 5x3x3 mm. The study materials were placed in a fresh fixative immediately after taking. According to the rule, the volume of the fixative was 25 times the volume of the pieces.

For the above purpose, we used a simple chemical fixative 10% neutral formalin. Fixation of the material was carried out at room temperature (at 22–24°C). To

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prepare thin histological sections, the materials were compacted by pouring into paraffin after preliminary dehydration. For dehydration, a battery of ethyl alcohols of increasing concentration (from 40° to 95°) was used. The starting point for the preparation of alcohols of various concentrations was 95° alcohol. For the preparation of alcohol of the required strength and in the right amount, table 1.3 was used [17,19,20,21].

Table 1

Scheme for the preparation of alcohol of the required strength

Overall 100 ml	In millilithers	
	95° alcahol	Distel. Water
40°	42	58
45°	47	53
50°	52	43
60°	63	37
70°	73	27
80°	83	17
90°	94	6

To obtain the absolute, we used white powder of calcined copper sulphate according to the method described by Yanin V.L. et al. (2015) [17,19]. The materials were dehydrated in cleanly washed and dried 250 ml jars. The alcohol level was higher than the material by more than 5 cm.

Pieces of materials were sequentially transferred from one alcohol solution to another, with exposure in each of them for 12 hours. Before transferring the material from one alcohol to another, the pieces were thoroughly dried with filter paper. A brief wiring diagram is shown in the following diagram (Table 2).

Table 2

Wiring diagram after fixation in formalin

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№	Steps	Duration
1.	40% ethanol	12 h.
2.	50% ethanol	12 h.
3.	60% ethanol	12 h.
4.	70% ethanol	12 h.
5.	80% ethanol	12 h.
6.	95% ethanol	12 h.
7.	100% (1) ethanol	6 h.
8.	100% (2) ethanol	6 h.
9.	Chloroform 1	30 min.
10.	Chloroform 2	30 min.
11.	Chloroform + parrafin (under 37°C)	30 min.
12.	Parrafin 1(under 56°C)	30 min.
13.	Parrafin 2 (under 56°C)	30 min.

After dehydration in alcohols, we poured the material into homogenized paraffin. For better cutting, we added wax: for 100 g of paraffin, 3-5 g of wax. In order to stain histological sections, deparaffinization was carried out in special histological cuvettes. Sections were kept for 1 minute in each of the solutions.

Sections were stained using the most common staining method, hematoxylin and eosin. The main dye is hematoxylin, which stains the nuclei of cells. And eosin is an acidic dye; it stains the cytoplasm of cells and some non-cellular structures. Sections were stained with hematoxylin prepared according to the Ehrlich method. To prepare eosin, 0.1 g of dye was dissolved in 100 ml of 95° alcohol. After successful staining, the sections were dehydrated in alcohols. Sections were enclosed in Canadian balsam. For a detailed study of the connective tissue and muscle tissue of the skin, the Van Gieson staining method was used. For this purpose, we used Weigert's iron hematoxylin and picrofuchsin as the acid stain [20,21].

RESULTS AND DISCUSSIONS

We have in these experiments used mainly mouse skin, since it is easy to obtain fresh, and easy to work with; but there are huge differences between normal and tattooed skin. Some features of histology of the two skins are described here.

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In histological studies of the skin of mouse (Figs.1,2.), it is studied several features of Epidermis. The dermal epidermal junction was regular with an absence of dermal papillae and epidermal ridges. Resting on a basement membrane is the stratum basale, consisting of a single layer of cuboidal to columnar cells with large, round to oval nuclei and 1 or 2 prominent nucleoli. The stratum spinosum had a thickness of 1 to 2 polyhedral cells, each with a single centrally placed oval nucleus and either 1 or 2 prominent nucleoli. The stratum granulosum appeared reduced, consisting of flattened cells with flattened oval nuclei and basophilic cytoplasmic granules. No stratum lucidum was present. The stratum corneum consisted of several layers of extremely flattened, a nucleate keratinised cells.

Dermis. The underlying dermis of the mouse skin presented as dense irregular connective tissue with large amounts of irregularly arranged collagen fibres interspersed with fibroblasts of which only the stained nuclei were discernible. Numerous pigment containing cells are homogeneously dispersed throughout the dermis. These cells had large, pale-staining nuclei that were frequently obscured by large amounts of brown pigment-containing granules.

The hypodermis, and was formed by a continuous unilocular adipose connective tissue layer, the panniculus adiposus. The large adipocytes were polyhedral in shape with a thin rim of cytoplasm surrounding a large unstained space with in the cell and their flattened nuclei eccentrically placed. Pigment containing cells were interspersed between the adipocytes and had the same morphology as those in the dermis. Large blood vessels were located in the basal region of the hypodermis while smaller vessels(capillaries) were found throughout this layer. Mast cells were positioned in close proximity to blood vessels and between adipocytes. Pigment containing cells, also present in the connective tissue, surrounded the striated skeletal muscle (panniculus carnosus) beneath the hypodermis.

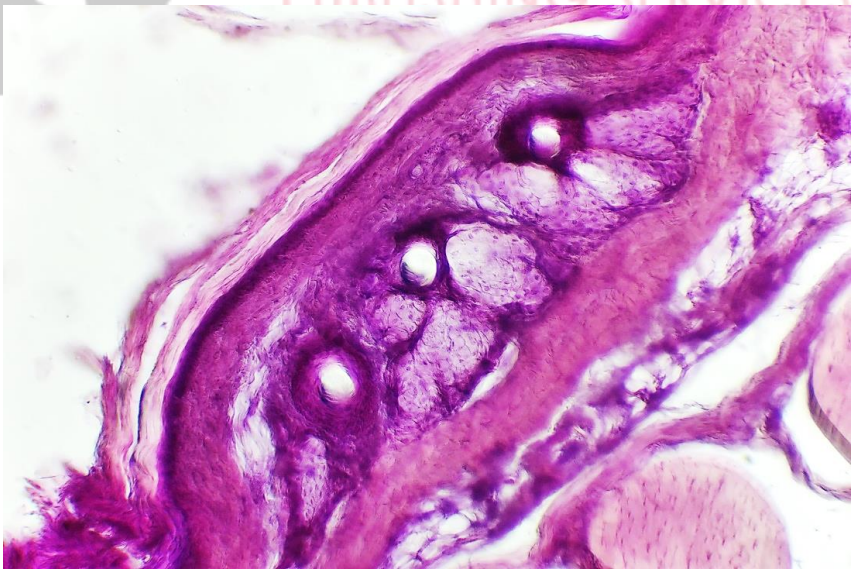

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Figure 1. Normal skin of mouse. Microscopic appearance. Epidermis is covered with a thin layer of keratin (1), epidermis (2), derma layer consisting of connective tissue (3), hair follicles (4), sebaceous glands (5), smooth muscle fibers (6). Stain hematoxylin-eosin. 20x20 ob

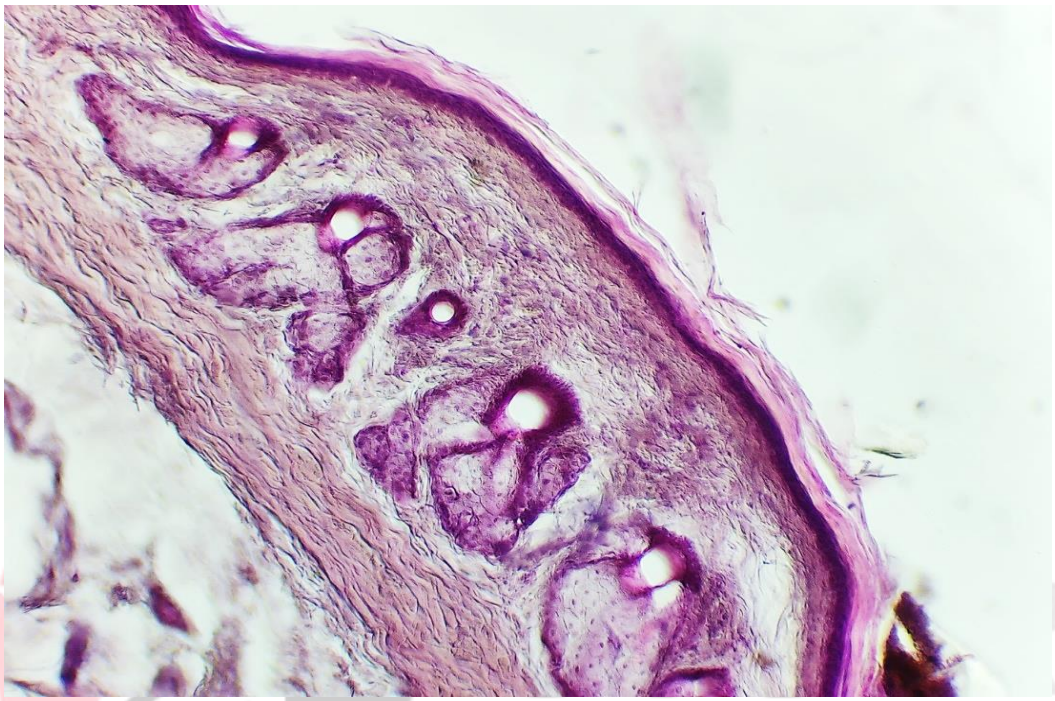

Figure 2. Normal skin of mouse. Microscopic appearance. Epidermis is covered with a thin layer of keratin (1), epidermis (2), derma layer consisting of connective tissue (3), hair follicles (4), sebaceous glands (5), smooth muscle fibers (6). Stain hematoxylin-eosin. 10x20 ob

Histomorphological differences between normal and tattooed mouse skin. Histological examination of mice tattooed with the 10% glycerol vehicle demonstrated essentially the same initial inflammatory response as mice tattooed with inks,suspensions of a pigment in the vehicle. Compared to normal non-tattooed skin (Fig. 2), tattooing resulted in acute trauma evidenced by epidermal necrosis, acute epidermal inflammation and dermal hemorrhage (Figs.3 and 4) as early as day 0.5 post-tattooing.

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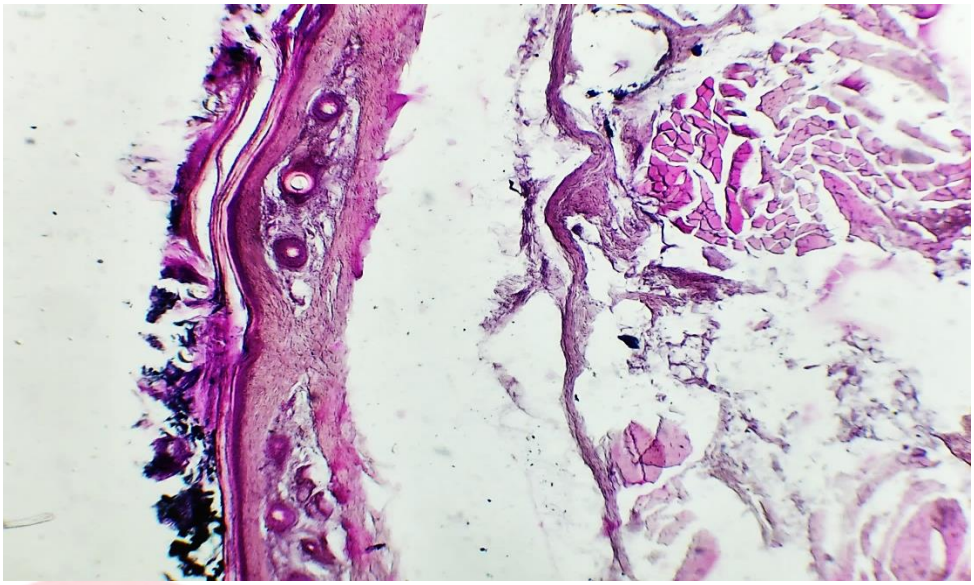
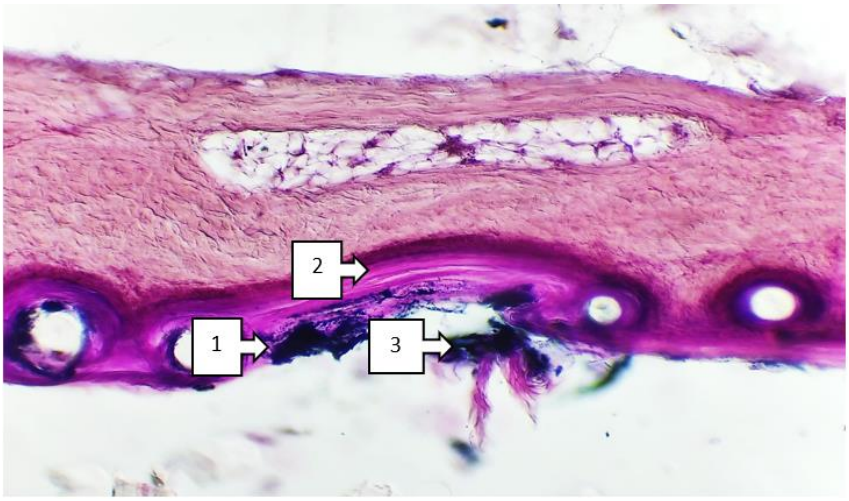


Figure 3. Tattooed skin of mouse. Microscopic appearance. Placement of pigments in the form of black granules (1) in the epidermis (2) epidermal inflammation (3)10x20 ob

Epidermal necrosis was present in all tattooed mice on days 0.5 and 1, decreasing in incidence by day 3 post-tattooing (Fig. 5). Acute epidermal inflammation persisted in 100% of tattooed animals from days 0.5 to 3, being completely absent from skin by day 7 (Fig. 3). Dermal hemorrhage was present exclusively on days 0.5 and 1 post-tattooing (Fig. 4).

The presence of pigment was confirmed visually in all groups of mice tattooed with inks, and not the 10% glycerol vehicle, at all time points tested (Figs.5,6).



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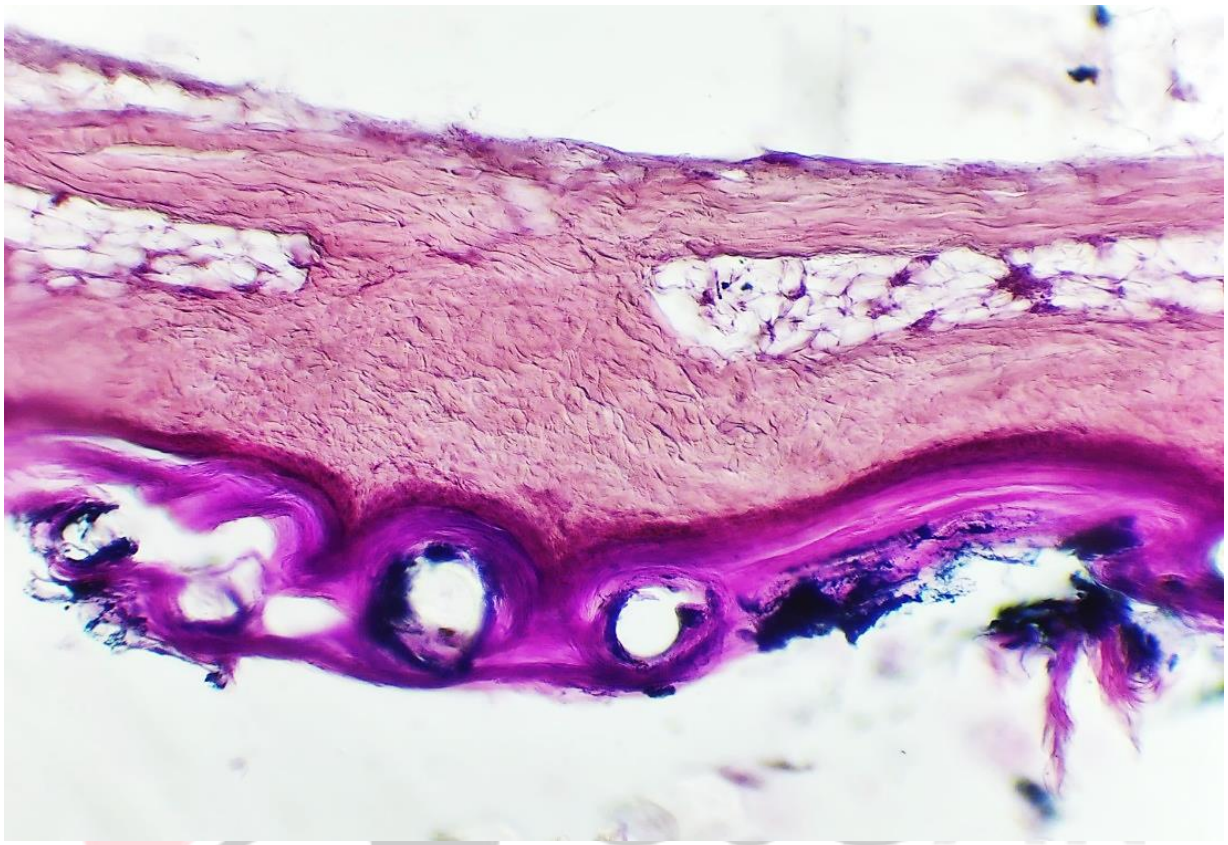

Figure 5-6. Tattooed skin of mouse. Microscopic appearance. Placement of pigments in the form of black granules (1) in the epidermis (2) epidermal necrosis (3)10x20

Regenerative epidermal hyperplasia (Figs.4) and chronic-active dermal inflammation (Fig. 4) were initiated in all tattooed mice from day 3 and remained active through day 14 post-tattooing. Dermal necrosis was present in the skin of 100% of tattooed mice on day 3 (Fig. 4) decreasing in incidence by day 7 and persisting exclusively in the CdS-tattooed animals until day 14 (data not shown)

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