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The ultrastructural effects of long-term use of henna on the albino rat skin

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Summary. Tattooing with henna is a routine practice in the Arab world. To the best of our knowledge, no previous studies have evaluated the adverse histological effects following henna tattooing on the ultrastructure of the skin. The objectives of this study were to diagnose the cytopathological alterations induced by commercial henna and to investigate the adverse role of henna when combined with sun ray on the skin. The skin of albino rats was tattooed with natural and black henna for three months, skin samples were examined by transmission electron microscope. In addition, the concentration of lead in henna samples was estimated by using atomic absorption spectrophotometry. The results expanded the understanding of the pathogenesis of henna-induced phytophotodermatitis. We hypothesized that henna-associated additives penetrated the epidermal barrier to gain access to the vascular dermis where the harmful ingredients became concentrated, leading to skin pathology through a dual mechanism. First, these ingredients became re-transported into the epidermis through vesicular trafficking leading to dermo-epidermal blistering and cytoplasmic vacuolization of the stratum basal cells. Following this, cytoplasmic vacuoles poured their content into the nuclei through continuities with the perinuclear cisterna, possibly leading to genetic mutation. The progression of keratinocytes into the next layers became associated with nuclear and cytoplasmic signs of apoptosis with subsequent phagocytosis in other epidermal cells, most probably keratinocytes. The second mechanism of injury was mediated through accumulation of inflammatory cells around capillaries in the dermis with the release of angiogenic and mitogenic mediators resulting in vasculopathy.

Key words: Henna, Albino rat, Keratinocytes, Cytoplasmic vacuoles, Lead

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Introduction

The role of henna tattoos has been described in allergic and lichenoid reactions (Taaffe et al., 1978; Clarke and Black, 1979; Winkelmann and Harris, 1979). Although henna, which is the dried and powdered leaf of Lawsonia inermis, is commonly used as paint or dye, various researchers have shown that commercially available henna contains many additives, such as p-phenylenediamine (PPD), and these additives might elicit the allergic reactions associated with commercial henna (Lestringant et al., 1999; Rubegni et al., 2000). Natural henna contains the agent lawsone, which is grayish green; all other colors obtained with henna are due to the addition of other synthetic agents (Natow, 1986). Some authors (Rubegni et al., 2000) reported a light microscopic histopathological finding of lichenoid dermatitis caused by allergic reaction due to the temporary henna tattoo, and they attributed these allergic reactions to the additive dyes present in commercial henna. Phytophotodermatitis is defined as a phototoxic reaction of the skin following contact with plant-derived substances and subsequent sunlight exposure, has also been reported with commericially available henna (Tunget al., 1994; Zhang and Zhu, 2011).

To the best of our knowledge, this is the first report which analyzed the ultra-structural cytological alterations and histological effects of henna tattooing. In this context, the objectives of the present study were to determine a reliable tool for early diagnosis of cytopathological alterations by using the advanced method of transmission electron microscope that might solve the skin problems associated with the use of henna. We compared the histopathological effects of both natural henna and black henna after tattooing on the skin. The second aim of this study was to evaluate the ultrastructural effects of henna application on skin when combined with solar irradiation.

Materials and methods

Henna samples

Henna samples (natural and black types) (Gadodia, khari Baoli, Delhi-110 006 India) were obtained from a local artisan at Qassim region; Saudi Arabia. After assaying Lead content in each sample, henna was used as tattoo on the skin of albino rats.

Animal groups

The study was approved by the Institutional Ethical Committee of Qassim University, and conducted on 20 mature albino rats. All were 6 months of age and without sex discrimination. The rats were classified into two groups (10 rats in each group):

Group - I: They were divided into two sub-groups; A and B (each of 5 animals); being subjected to natural and black henna respectively. Fur from the dorsum of used animals was manually epilated 72 hours before the experimental tattooing with henna in order to avoid trauma artifacts. The skin of the right epilated side was soaked with a water-based emulsion of the corresponding type of henna twice a week for three months, while the left epilated side was used as control (wetted only with water). Animals were kept in shade.

Group - II: was managed as group–I, but immediately after henna application; the animals were exposed to solar irradiation (during January/February) for 30 minutes at 11 a.m. in the city of Buraidah at the central zone of Saudi Arabia.

Atomic absorption spectrophotometry

Atomic Absorption Spectrophotometry technique (Kumhomkul and Panich-Pat, 2013) (Perkin-Elmer Model 400, Shelton, CT, USA) was used to determine the lead content present in natural and commercial henna samples.

Transmission electron microscopy

By the end of the experiment, animals were sacrificed under ketamine anesthesia (intramuscular injection of 90 mg/kg) and skin biopsies (fragments of about 1 mm^3) were obtained, immersed in a fixative formed of 2.5% glutaraldehyde in 0.1 M Cacodylate buffer (pH 7.3) for four hours at 4°C, then post fixed in 1% Osmium tetroxide in 0.1 M Cacodylate buffer (pH 7.3) for 2 hours. The specimens were dehydrated in ascending grades of ethanol. After immersion in propylene oxide (three times for 10-minutes each), the samples were impregnated overnight in a mixture (1:1) of propylene oxide and Epon-812 resin (Spa- USA) to be lastly embedded in Epon-812. Semithin sections (0.5 micron thickness) were stained with toluidine blue to select the proper sites for ultrathin sections (60-80 nm thickness). The ultrathin sections were mounted on formvar coated copper slot grids, and stained with 2% uranyl acetate and 1% lead citrate (Hayat, 1989). The grids were examined by a JTEM 1010 (Jeol - Japan).

Results

Lead estimation in natural and black henna

Atomic Absorption Spectrophotometry was used to analyze the lead content present in henna samples.The results showed that natural henna contained 26 μg/gm, whereas black henna contained 440 μg/gm.

Electron microscopic results

The epidermis of the control rats was principally made up of keratinocytes where tonofilaments are inserted at sites of intercellular contact and with the basement membrane. Occasional basal cells were found completely devoid of cytoplasmic filaments, while mitochondria showed evidence of internal membranes and scattered dense melanin granules could be seen clearly. The latter seemed to be elaborated into the cytoplasm of the neighboring keratinocytes to aggregate at the supra-nuclear region forming a cap (Fig. 1a). Multiple layers of cornified non-nucleated scales formed the stratum corneum; where corneocytes seemed still compacted together and adherent to the surface of the epidermis through multiple desmosomal junctions, in addition to end-to-end interlocking sites. Stratum disjunctum was represented by one layer of keratin-filled corneocytes that had completely lost their desmosomal contact with the surface of the epidermis (Fig. 1b). The dermis in control rats contained collagenous fibers and exhibited occasional mast cells as characterized by cytoplasmic granulation and irregular vacuolization (Fig. 1c).

In the group of animals in which natural henna was applied and kept in shadow, the cytoplasm of keratinocytes exhibited only clear peri-nuclear regions (Fig. 2a). Post-staining exposure to sun rays of that group led to an appearance of some cytoplasmic vacuolization around the nuclei of keratinocytes in stratum spinosum with melanin accumulation at the supra-nuclear region and occasional dilatation of the

peri-nuclear cisternae (Fig. 2b).

In the painted animals with black henna and kept in shadow, the ultra-structural findings revealed marked vacuolization in the cytoplasm of keratinocytes and in the papillary layer of dermis; many of these cytoplasmic vacuoles had encroached the nuclear envelope and even gained access to the peri-nuclear cistern (Fig. 3a). Although most nuclei were spared, some of them appeared condensed and exhibited large vacuoles (Fig. 3b).

Post-staining exposure to actinic rays in the black henna-treated group resulted in more dramatic ultra-structural changes; projection of dermal micropapillae and extensive cytoplasmic vacuolization of keratinocytes were evident, accompanied by the appearance of large vacuolar structures inside the condensed nuclei. Crumbling of some keratinocytes with subsequent phagocytosis in the stratum spinosum layer was an inevitable consequence (Fig. 4). Most layers of corneocytes belonged to the stratum disjunctum (Fig. 5). Concomitant with these epidermal changes, heavy mononuclear cellular infiltration (about 12-15 cells observed in the electron micrograph) into the dermis was noticed; these cells were identified as mast cells because of the granular appearance of their cytoplasm (Fig. 6a), while in the control dermis (Fig. 1c) an occasional mast cell was seen. Structural defects were also detected in capillaries; the capillary endothelial cells revealed large cytoplasmic vacuoles and many endothelial outgrowing folds, that divided the capillary lumen into many spaces (Fig. 6b).

Discussion

There are only few reports on skin sensitization by the use of natural henna (Ramírez-Andreo et al., 2007), but now henna preparations are fortified with various herbs or synthetic materials in order to give it a stronger color. The added materials are suspected to induce skin sensitization as these materials are very rich in heavy metals such as Mercury and Lead salts (Lekouch et al., 2001). In the present study, digested henna samples were analyzed for lead. Our results showed that lead concentration was significantly higher in black

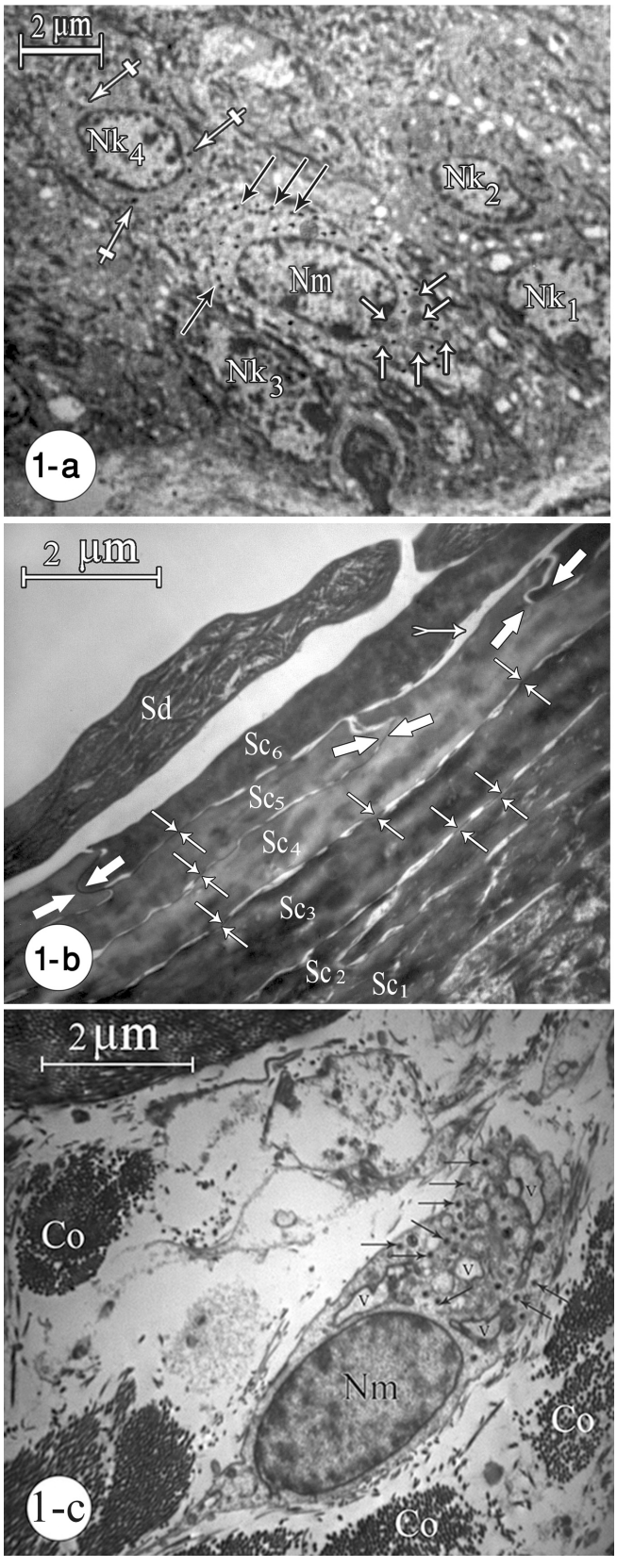

Fig. 1. Electron micrographs of control epidermis **(a, b)**. **a.** Shows keratinocytes (NK1-4) that are rich in tonofilaments and melanin granules (crossed arrows); the cell (Nm) is rich in mitochondria (white arrows) and melanin granules (black arrows). **b.** Shows that stratum corneum is formed of compacted layers of corneocytes (Sc_{1-6}) that have end-to-end interlocking contacts (double thick arrows) with many locations of desmosomal junctions (double thin arrows). At the most superficial layer, an irregular dilatation (forked arrow) of the intercellular space appears where desmosomal contacts are lost. Stratum disjunctum is represented by a layer of corneocytes (Sd) that showed fibrillar appearance of its keratin content. **c.** Shows control dermis which contains an occasional elongated mast cell having a vesicular nucleus (Nm) and exhibiting some cytoplasmic granules (arrows) and irregular vacuoles (V). Notice; collagen bundles (Co).

(commercial) henna as compared to the natural henna. These findings clearly indicated that lead-rich additives were added in commercial preparations of henna. A recent study using Inductively Coupled Plasma Optical Emission Spectroscopy (Jallad and Espada-Jallad, 2008), reported that lead amounted to 2.29 ppm and 65.98 ppm in natural and black henna respectively, also United States Food and Drug Administration set an action level (enforceable) of 0.5 μg/ml or 0.5 ppm for lead in products for use by infants and children. Previous studies have shown that inorganic lead was absorbed through the skin and was rapidly distributed through the body leading to abnormal blood lead levels (Florence et al., 1988).

Black henna is obtained from natural henna after the addition of other compounds, among them para-phenylenediamine (PPD), which triggers allergic contact dermatitis (Matulich and Sullivan, 2005). Sensitization to PPD is important for two reasons. First, its ubiquity—it is present in substances such as hair dye, eye shadow, plastics, rubber, printing ink, and developing fluid (Arranz Sánchez et al., 2005). The other reason, it has a cross-reactivity with other structurally similar classes of compounds, including the azo-dyes (used in textiles), the sulfonamides, glucose-lowering agents, and p-aminobenzoic acid (PABA); a common component of sunscreens and PABA-derived local anesthetics (Di Prisco et al., 2006; Ramírez-Andreo et al., 2007).

During this study, we noticed that the lateral borders between corneocytes were generally interdigitated. Desmosomal junctions were usually encountered between the keratinocytes, especially at the wide opposing surfaces and occasionally along the side-to-side interlocking surfaces (Glenn et al., 2000; Bouwstra and Honeywell-Nguyen, 2002).

Little is known about the potential risks of toxicity by contact with henna-additives. Theories of transdermal particle delivery suggested that skin structure cannot allow the penetration of materials larger than 600 Da (Baroli et al., 2007). Several methods have now been assessed to increase the permeation rate across the stratum corneum (Friedland and Buchel, 2000; Barry, 2001; Bouwstra and Honeywell-Nguyen, 2002; Güereña-Burgueño et al., 2002; Menon et al., 2003; Glenn et al., 2007).

The pilosebaceous unit (hair follicle, hair shaft and sebaceous gland) provides a follicular delivery route that bypasses intact stratum corneum; the sebaceous gland

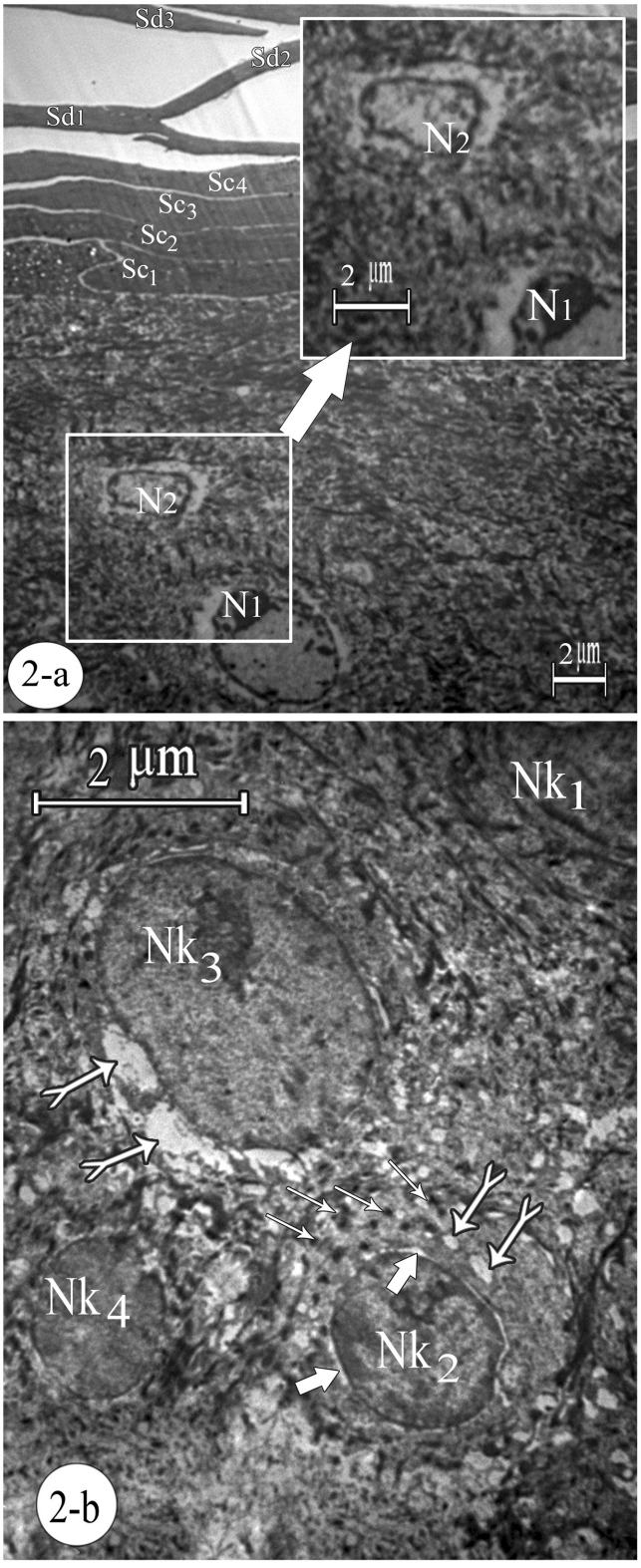

Fig. 2. Photographs of natural henna-painted animals. **a.** For animals kept in shadow shows that the nuclei (N_{1-2}) of keratinocytes in the stratum spinosum are vesicular but the perinuclear cytoplasm appears free from tonofilaments. Notice that stratum corneum is formed of many layers of compacted corneocytes (Sc_{1-4}) while desquamating flakes of stratum disjunctum are few (Sd_{1-3}). Sun co-exposure **(b)** shows that keratinocytes (Nk_{1-4}) had aggregations of melanin granules (thin arrows) with appearance of small cytoplasmic vacuoles (forked arrows) and occasional dilatation of the perinuclear cisternae (thick arrows).

cells are more permeable than corneocytes and thus drugs can reach the dermis by entering the follicle, passing through the sebaceous gland or penetrating the epithelium of the follicular sheath (Baroli et al., 2007).

The aim of this study was to examine skin changes in henna tattooing and post-exposure by sun rays using the advanced method of transmission electron microscope. We hypothesized that this technique would detect henna-induced tissue pathology in early and late induction. In previous literature, it was mentioned that

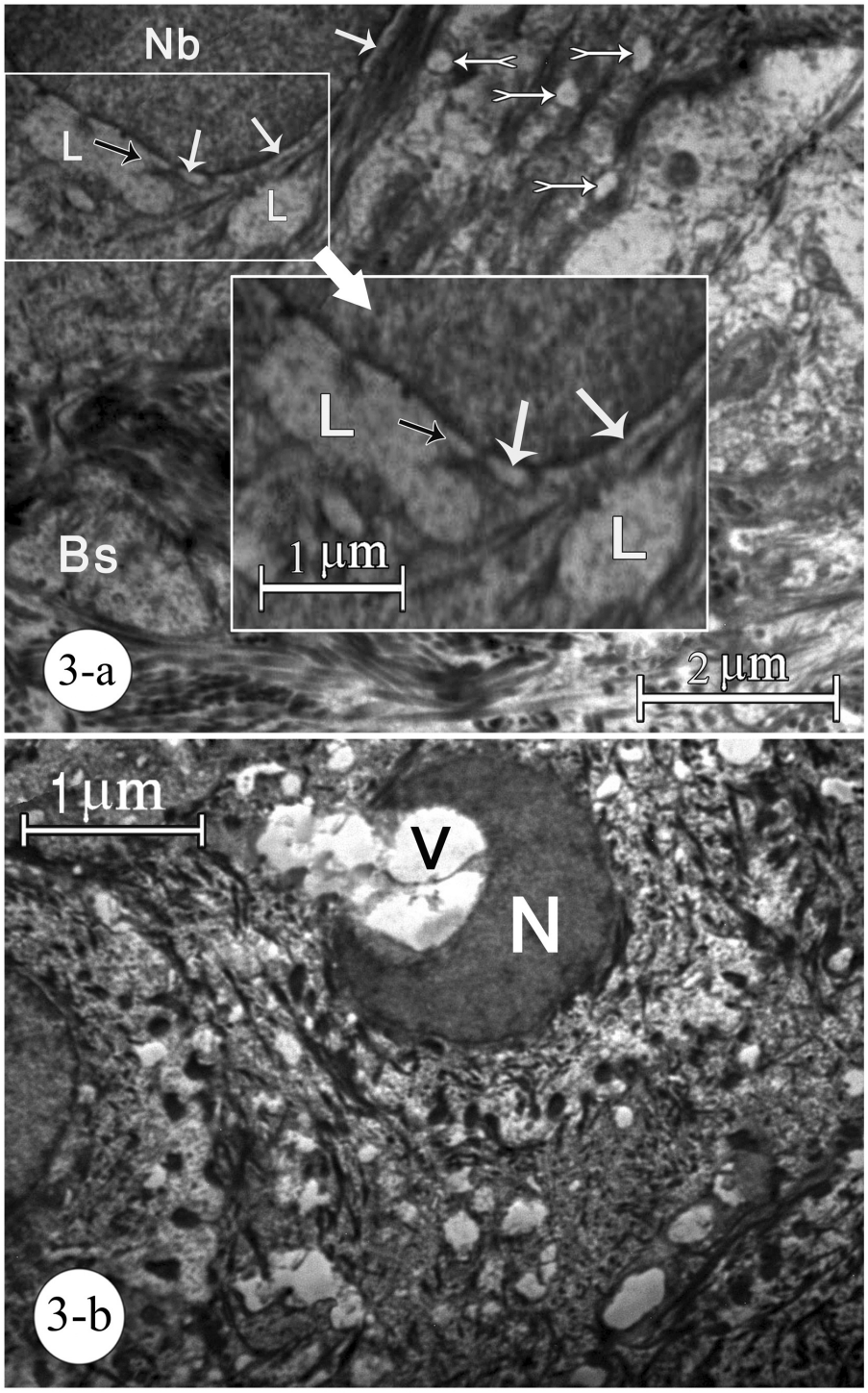

Fig. 3. Electron micrographs from black henna-painted animals and kept in shadow. **a.** Shows a portion of a basal keratinocyte (Nb) that has many cytoplasmic small vesicles (forked arrows) and large vacuoles (L). A dermal basal space (Bs) is detected at the left lower quarter. The magnified part in the inset clarifies that a large cytoplasmic vacuole had communicated (black arrow) with the perinuclear cistern (thin white arrows). **b.** Reveals the appearance of a large vacuole (V) inside the condensed nucleus (N) of a cell in stratum spinosum.

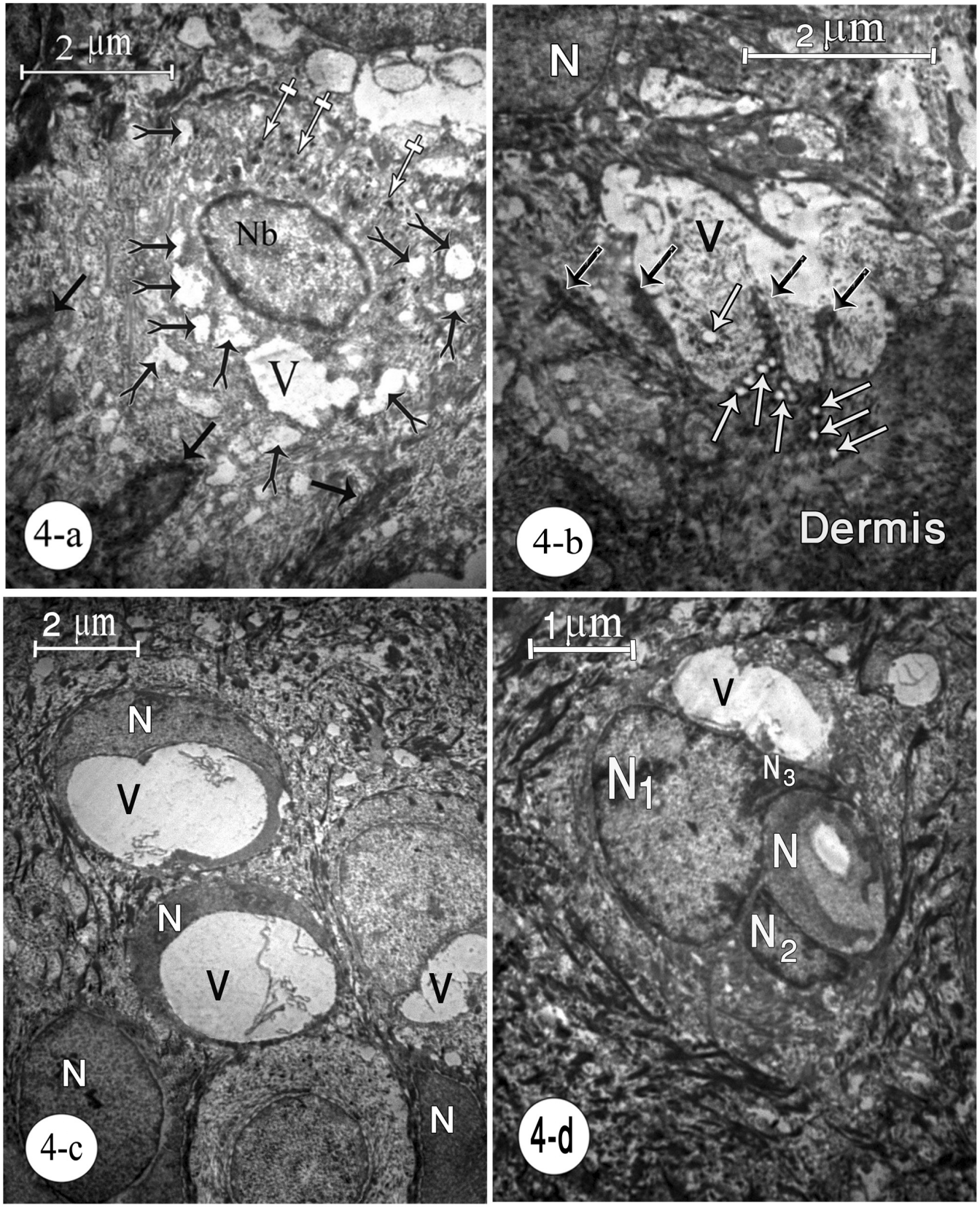

Fig. 4. Micrographs of group-II- black henna-painted animals. Variable-sized cytoplasmic vesicles (forked arrows) and large vacuoles (V) appear in keratinocytes of the basal layer (**a and b**) together with dermal micropapillae (black arrows in **a and b**) that become associated with a dermal vesicular traffic (white arrows in **b**) and heavy supra-nuclear accumulation of melanin granules (crossed arrows in **a**). The nuclei (N) of keratinocytes in the spinous layer appear condensed and are occupied with large vacuolar structures (V in **c**) and sometimes are engulfed (**d**) inside healthy keratinocytes (N_{1-3}).

erythema was observed only 48 hours after handling the peel juice of Tahiti lemon which contains psoralens. These changes over time indicated that the interaction of sunlight and psoralens triggered cell death, which became detectable by light microscopy for many hours later, and took even longer to manifest clinically. This delay was in agreement with clinical (Almeida et al., 2008) and experimental experiences (Gonçalves et al., 2005; Almeida et al., 2008).

In this study, dermo-epidermal junctional blistering in black henna-treated phytophotodermatitis was observed. In cases of recessive dystrophic epidermolysis bullosa with junctional blisters, the structural defects of hemidesmosomes were considered to play the most important role in the pathogenesis of junctional blisters (Hashimoto et al., 1976). Probably the cell membrane proteins involved in the adhesion of prekeratin tonofilaments in the desmosomal plaque and keratin are more sensitive to the photoproduct, made when sunlight and henna additives (PPD) interact (Almeida et al., 2008). These protein lesions led to keratinocyte death and blistering. This information is in contrast to the previous concept, which states that nuclear changes could be the cause of cell necrosis in phototoxic reactions (Tunget et al., 1994).

The results of the present study underscore the excellent permeability of the epidermal intercellular spaces for small particles. In guinea pigs, it was reported that the internalization of thorotrast marker is accomplished by means of single membrane-limited phagosomes which transfer the marker into the interior of the keratinocytes (Wolff and Hönigsmann, 1971)

Mild degenerative signs appeared in some keratinocytes after application of black henna. However, in samples taken with sun co-exposure, these signs became dramatically increased even before these cells became engulfed inside others. Apoptosis is defined as a process of programmed cell death and it is a widespread developmental event characterized by morphological changes in cytoplasmic and nuclear structures (Wyllie et al., 1980; Lockshine and Zakeria, 1991; Lin et al., 2013). In the present study, we demonstrated extensive cytoplasmic vacuolization of keratinocytes, huge nuclear autolytic vacuoles inside the affected keratinocytes of

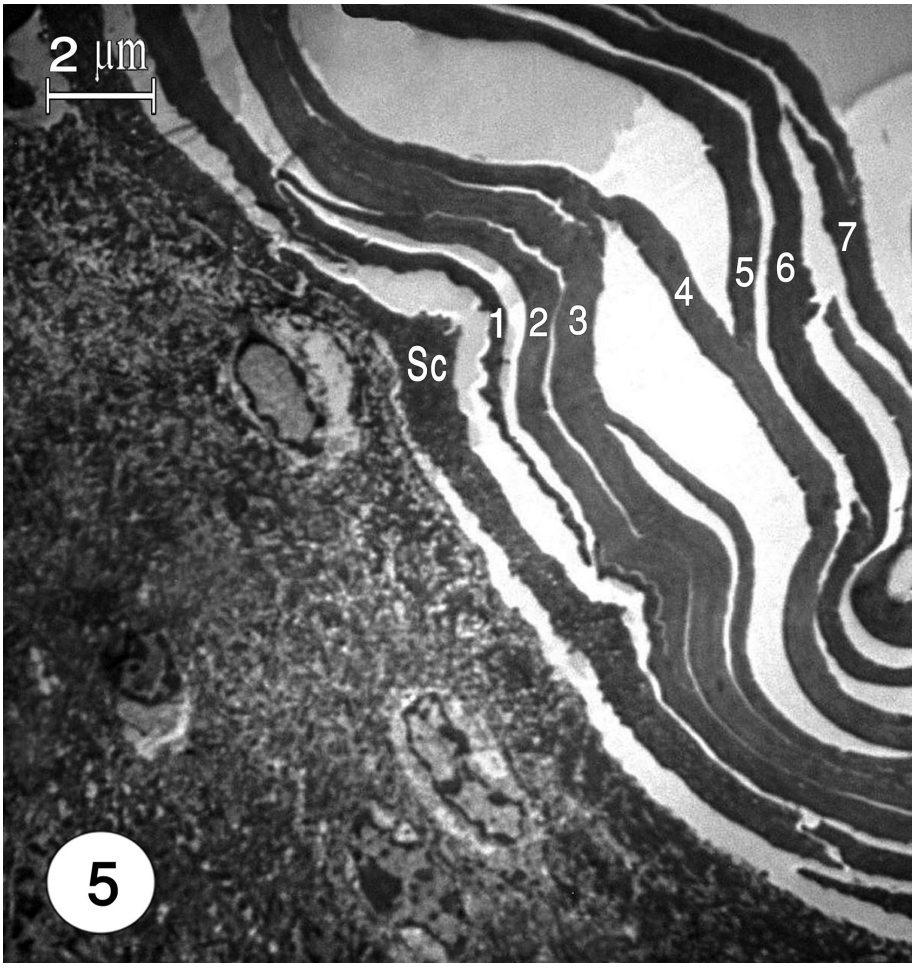

Fig. 5. A micrograph through the superficial layer of epidermis in group-II- black henna-painted animal. Most layers of corneocytes belonging to the stratum disjunctum have already desquamated (1-7), while only one layer (Sc) is still adherent to the epidermis to represent the stratum corneum.

unknown contents which needs further immunohistochemical examination. Nuclear envelopathies refer to disorders caused by mutations in the genes encoding nuclear envelope proteins, such as A-type lamins (LMNA) and emerin (Park et al., 2009). This "nucleophagy" is intended to clean up nuclear waste produced by nuclear damage. Lamins form a protein meshwork of nuclear lamina at the nucleoplasmic side of inner nuclear membrane and have an important role in the maintenance of nuclear architecture, so the mutations

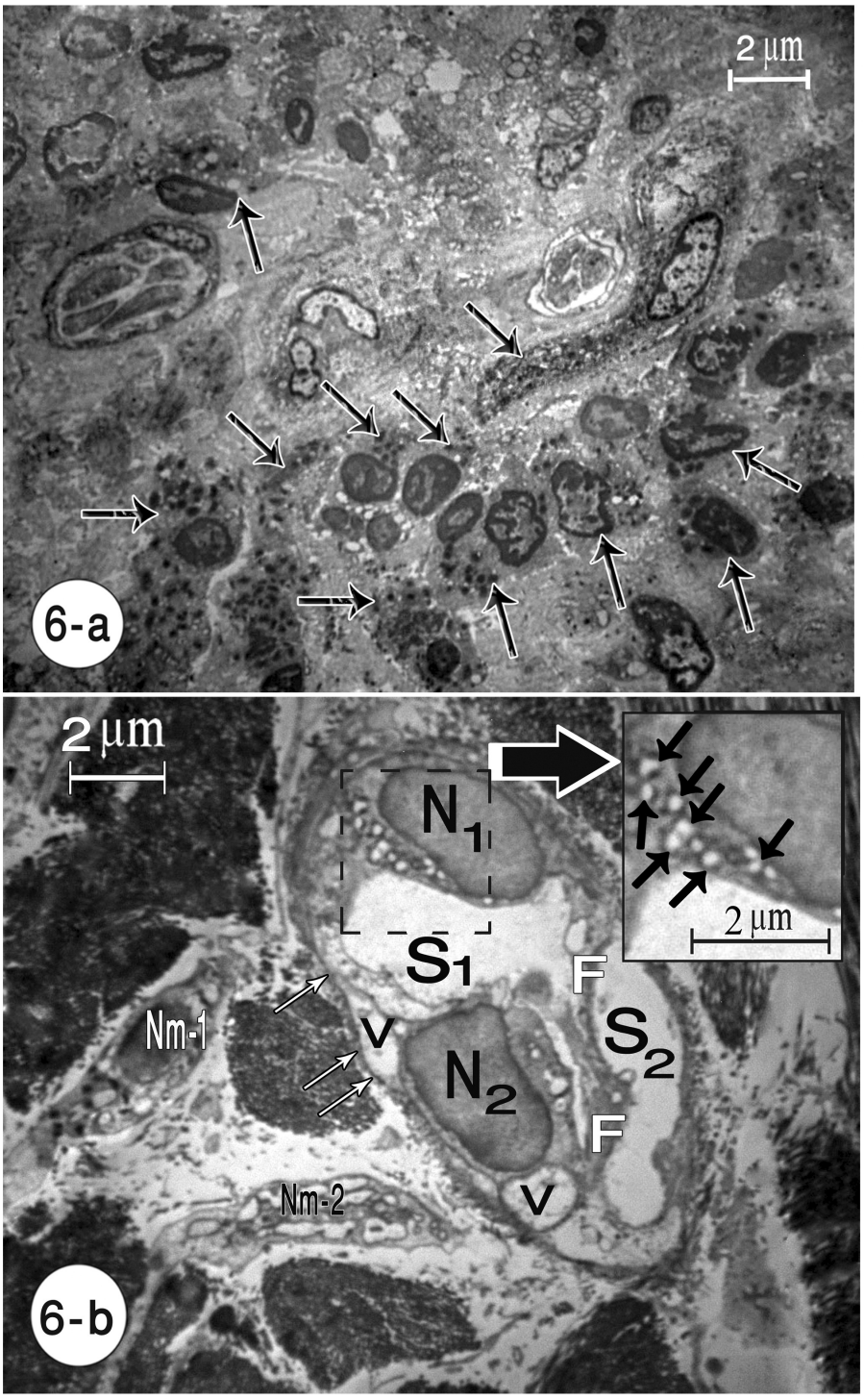

Fig. 6. Electron micrograph of dermis of group-II- black henna-painted animal. A heavy mononuclear cellular infiltrate (about 12 cells) is noticed in **a**; most of the cells (arrows) appear irregular or even elongated in shape with prominent granules in their cytoplasm. Compare with the control dermis (**Fig. 1c**) which contains an occasional mast cell. In **b**, two mast cells (Nm_{1-2}) with granules and vacuoles in their cytoplasm are associated with a capillary that is lined with endothelial cells containing vesicular nuclei (N_{1-2}), notice appearance of large vacuoles (V) between the endothelial cells and their basement membrane (white arrows) while many vesicles (black arrows) appear within the capillary endothelium. Endothelial microfolds (F) fuse inside the capillary lumen dividing it into two spaces (S_{1-2}).

in LMNA are thought to the cause of nuclear membrane fragility. This phenomenon is expected especially in cells which are constantly subjected to repeated mechanical stress (Broers et al., 2004; Lammerding et al., 2004).

We observed that dying apoptotic keratinocytes were phagocytized by other epidermal cells that apparently had many nuclear lobes. Although keratinocyte phagocytosis was rare in the system studied, keratinocyte phagocytic capability was reported by other authors (Wolff and Konrad, 1972; Maccallum and Scalett, 1973; Hashimoto et al., 1976; Enomoto et al., 2011; Sayedyahossein et al., 2012). Occasionally, nuclear indentations may cause one nucleus to be sectioned many times in the same plane so that the cell may falsely appear to contain many nuclear lobes.

We noticed dermal aggregation and degranulation of mast cells in lesions induced by black henna, particularly after photo-sensitization. The number of mast cells greatly increased in the cutaneous lesion of allergic dermatitis (Weng et al., 2012). Ultraviolet-B radiation activates mast cells; neuropeptide is released from neural c-fibers that in turn trigger histamine secretion from mast cells, leading to suppression of the cellular immune system (Fisher and Kripke, 1977; Kripke, 1984; Grimbaldeston et al., 2002; Matsumura and Ananthaswamy, 2002; Ch'ng et al., 2006).

In phytophotodermatitis induced by black henna in this study, mast cells accumulated around dermal capillaries which probably led to changes in the capillary structure manifested as capillary enlargement and the induction of endothelial uneven growth. Mast cells are the major source of vascular endothelial growth factor (VEGF) in basal cell carcinoma and malignant melanoma; it is one of the most potent angiogenic factors, which also induces leakage of other angiogenic factors across the endothelial cell membrane into the matrix (Reed et al., 1995; Ugurel et al., 2001; Ribatti et al., 2003). The increased amount of the growth factors might be the cause of development of capillary vasculopathy, manifested as capillary enlargement and induction of endothelial uneven microfold growth, which divided the lumen into many spaces, and might lead to abnormal blood flow in the capillaries. From previous studies, local vascular endothelial growth factor–A (VEGF-A) concentration and blood flow were known to modulate the structure of angiogenic vessels (Ozawa et al., 2004; Rissanen et al., 2005). Systemic toxicity of black henna has been reported in certain African countries (De Groot, 2013).

Concluding remarks

The present electron microscopic study showed that the henna-associated additives in black henna, especially after exposure to actinic rays, resulted in pronounced pathological ultra-structural changes. Allergic contact dermatitis with henna and its additives may induce progress of phytophotodermatitis and hypertrophic scar. Therefore, it is important for health professionals to take steps to increase the awareness of toxicity induced by commercially available henna. This approach seems to be the only reliable way of ending or at least reducing the use of commercial henna, especially when children are involved.

References

Almeida H.L. Jr, Sotto M.N., Castro L.A. and Rocha N.M. (2008). Transmission electron microscopy of the preclinical phase of experimental Phytophotodermatitis. Clinics (Sao Paulo) 63, 371-374.

Arranz Sánchez D.M., Corral de la Calle M., Vidaurrazaga Díaz de Arcaya C., de Lucas Laguna R. and Díaz R. (2005). Risks of black henna tattoos. An. Pediatr. (Barc) 63, 448-452.

Baroli B., Ennas M.G., Loffredo F., Isola M., Pinna R. and López-Quintela M.A. (2007). Penetration of metallic nanoparticles in human full-thickness skin. J. Invest. Dermatol. 127, 1701-1712.

Barry B.W. (2001). Novel mechanisms and devices to enable successful transdermal drug Delivery. Eur. J. Pharmaceut. Sci. 14, 101-114.

Bouwstra J.A. and Honeywell-Nguyen P.L. (2002). Skin structure and mode of action of vesicles. Advanced Drug Delivery Rev. 54 (Suppl. 1), S41-S55.

Broers J.L., Peeters E.A., Kuijpers H.J., Endert J., Bouten C.V., Oomens, Baaijens F.P. and Ramaekers F.C. (2004). Decreased mechanical stiffness in LMNA-/- cells is caused by defective nucleo-cytoskeletal integrity: implications for the development of laminopathies. Hum. Mol. Genet. 13, 2567-2580.

Ch'ng S., Wallis R.A., Yuan L., Davis P.F. and Tan S.T. (2006). Mast cells and cutaneous malignancies. Mod. Pathol. 19, 149-159.

Clarke J. and Black M.M. (1979). Lichenoid tattoo reactions. Br. J. Dermatol. 100, 451-454.

De Groot A.C. (2013). Side-effects of henna and semi-permanent 'black henna' tattoos: a full review. Contact Dermatitis 69, 1-25.

Di Prisco M.C., Puig L. and Alomar A. (2006). Contact dermatitis due to para-phenylenediamine on a temporal tattoo with henna: cross reaction to azoic dyes. Invest. Clin. 47, 295-299.

Enomoto A., Yoshihisa Y., Yamakoshi T., Ur Rehman M., Norisugi O., Hara H., Matsunaga K., Makino T., Nishihira J. and Shimizu T. (2011). UV-B radiation induces macrophage migration inhibitory factor-mediated melanogenesis through activation of protease-activated receptor-2 and stem cell factor in keratinocytes. Am. J. Pathol. 178, 679-687.

Fisher M. and Kripke M. (1977). Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc. Natl. Acad. Sci. USA 74, 1688-1692.

Florence T.M., Lilley S.G. and Stauber J.L. (1988). Skin absorption of lead. Lancet 2, 157-158.

Friedland J.A. and Buchel E.W. (2000). Skin care and the topical treatment of aging skin. Clin. Plastic Surg. 27, 501-506.

Glenn G.M., Taylor D.N., Li X, Frankel S., Montemarano A. and Alving C.R. (2000). Transcutaneous immunization: a human vaccine delivery strategy using a patch. Nat. Med. 6, 1403-1406.

Glenn G.M., Villar C.P., Flyer D.C., Bourgeois A.L., McKenzie R., Lavker R.M. and Frech S.A. (2007). Safety and immunogenicity of an enterotoxigenic *Escherichia coli* vaccine patch containing heat-labile toxin: Use of skin pretreatment to disrupt the stratum corneum. Infect. Immun. 75, 2163-2170.

Gonçalves N.E., de Almeida H.L. Jr, Hallal E.C. and Amado M. (2005).

Experimental phytophotodermatitis. Photodermatol. Photoimmunol. Photomed. 21, 318-321.

Grimbaldeston M.A., Skov L., Finlay-Jones J.J. and Hart P.H. (2002). Increased dermal mast cell prevalence and susceptibility to development of basal cell carcinomas in humans. Methods 28, 90-96.

Güereña-Burgueño F., Hall E.R., Taylor D.N., Cassels F.J., Scott D.A., Wolf M.K., Roberts Z.J., Nesterova G.V., Alving C.R. and Glenn G.M. (2002). Safety and immunogenicity of a prototype enterotoxigenic *Escherichia coli* vaccine administered transcutaneously. Infect. Immun. 70, 1874-1880.

Hashimoto I., Gedde-Dahl T. Jr, Schnyder U.W. and Anton-Lamprecht I. (1976). Ultrastructural studies in Epidermolysis Bullosa Hereditaria. IV- Recessive dystrophic types with junctional blistering. (Infantile or Herlitz-Pearson type and adult type). Arch. Dermatol. Res. 257, 17-32.

Hayat M.A. (1989). Principles and techniques of electron microscopy. Biological applications. 3rd ed. CRC Press, New York. pp, 24-74.

Jallad K.N. and Espada-Jallad C. (2008). Lead exposure from the use of Lawsonia inermis (Henna) in temporary paint-on-tattooing and hair dying. Sci. Total Environ. 397, 244-250.

Kripke M. (1984). Immunological unresponsiveness induced by ultraviolet radiation. Immunol. Rev. 10280, 87-102.

Kumhomkul T. and Panich-Pat T. (2013). Lead accumulation in the straw mushroom, *Volvariella volvacea*, from lead contaminated rice straw and stubble. Bull. Environ. Contam. Toxicol. 91, 231-234.

Lammerding J., Schulze P.C., Takahashi T., Kozlov S., Sullivan T., Kamm R., Stewart C.L. and Lee R.T.D. (2004). Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. J. Clin. Invest. 113, 370-378.

Lekouch N., Sedki A., Nejmeddine A. and Gamon S. (2001). Lead and traditional Moroccan pharmacopeia. Sci. Total Environ. 280, 39-42.

Lestringant G.G., Bener A. and Frossard P.M. (1999). Cutaneous reactions to henna and associated additives. Br. J. Dermatol. 141, 598-600.

Lin Y., Sun X., Qiu L., Huang Q., Fang C., Ye T., Kang M., Shen H. and Dong S. (2013). Exposure to bisphenol A induces dysfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma (INS-1) cells. Cell Death Dis. 17, e460.

Lockshine R.A. and Zakeria Z. (1991). Programmed cell death and apoptosis. In: Current communication in cell and biology. Chap. 3, apoptosis: The molecular basis of cell death. Tomei L.D. and Cope F.O. (eds). Cold Spring Harbor Lab. Press. New York. pp 47-60.

Maccallum D.K. and Scalett L.J. (1973). Possible phagocytosis by keratinocytes. Ultrastructural evidence from the study of human oral mucosa in organ culture. Arch. Oral Biol. 18, 449-455.

Matsumura Y. and Ananthaswamy H. (2002). Molecular mechanisms of photocarcinogenesis. Front. Biosci. 7, d765-d783.

Matulich J. and Sullivan J. (2005). A temporary henna tattoo causing hair and clothing dye allergy. Contact Dermatitis. 53, 33-36.

Menon G.K., Kollias N. and Doukas A.G. (2003). Ultrastructural evidence of stratum corneum permeabilization induced by photomechanical maves. J. Invest. Dermatol.121, 104-109.

Natow A.J. (1986). Henna. Cutis 38, 21.

Ozawa C.R., Banfi A., Glazer N.L., Thurston G., Springer M.L., Kraft P.E., McDonald D.M. and Blau H. M. (2004). Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. J. Clin. Invest. 113, 516-527.

Park Y.E., Hayashi Y.K., Bonne G., Arimura T., Noguchi S., Nonaka I. and Nishino I. (2009). Autophagic degradation of nuclear components in mammalian cells. Autophagy 5-6, 795-804

Ramírez-Andreo A., Hernández-Gil A., Brufau C., Marín N., Jiménez N., Hernández-Gil J., Tercedor J. and Soria C. (2007). Allergic contact dermatitis to temporary henna tattoos. Actas Dermosifiliogr. 98, 91-95.

Reed J., Albino A. and McNutt N. (1995). Human cutaneous mast cells express b-FGF. Lab. Invest. 72, 215-222.

39. Ribatti D., Vacca A., Ria R., Marzullo A., Nico B., Filotico R., Roncali L. and Dammacco F. (2003). Neovascularization, expression of fibroblast growth factor-2, and mast cells with tryptase activity increase simultaneously with pathological progression in human malignant melanoma. Eur. J. Cancer 39, 666-674.

Rissanen T. T., Korpisalo P., Markkanen J.E., Liimatainen T., Ordén M.R., Kholová I., de Goede A., Heikura T., Gröhn O.H. and Ylä-Herttuala S. (2005). Blood flow remodels growing vasculature during vascular endothelial growth factor gene therapy and determines between capillary arterialization and sprouting angiogenesis. Circulation 112, 3937-3946.

Rubegni P., Fimiani M., de Aloe G. and Andreassi L. (2000). Lichenoid reaction to temporary tattoo. Contact Dermatitis 42, 117-118.

Sayedyahossein S., Nini L., Irvine T.S. and Dagnino L. (2012). Essential role of integrin-linked kinase in regulation of phagocytosis in keratinocytes. FASEB J. 26, 4218-4229.

Taaffe A., Knight A.G. and Marks R. (1978). Lichenoid tattoo hypersensitivity. BMJ 1, 616-618.

Tunget C.L., Turchen S.G., Manoguerra A.S., Clark R.F. and Pudoff D.E. (1994). Sunlight and the plant: a toxic combination: severe phytophotodermatitis from *Cneorium dumosun*. Cutis 54, 400-402.

Ugurel S., Rappl G., Tilgen W. and Reinhold U. (2001). Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J. Clin. Oncol. 19, 577-583.

Weng Z., Zhang B., Asadi S., Sismanopoulos N., Butcher A., Fu X., Katsarou-Katsari A., Antoniou C. and Theoharides T.C. (2012). Quercetin is more effective than cromolyn in blocking human mast cell cytokine release and inhibits contact dermatitis and photosensitivity in humans. PLoS One 7, e33805.

Winkelmann R.K. and Harris R.B. (1979). Lichenoid delayed hypersensitivity reactions in tattoos. J. Cutan. Pathol. 6, 59-65.

Wolff K. and Hönigsmann H. (1971). Permeability of the epidermis and the phagocytic activity of keratinocytes: Ultrastructural studies with thorotrast as a marker. J. Ultrastruct. Res. 35, 176-190.

Wolff K. and Konrad K. (1972). Phagocytosis of latex beads by epidermal keratinocytes in vivo. J. Ultrastruct. Res. 39, 262-280.

Wyllie A.H., Kerr J.F. and Currie A.R. (1980). Cell death: the significance of apoptosis. Int. Rev. Cytol. 68, 251-306.

Zhang R. and Zhu W. (2011). Phytophotodermatitis due to Chinese herbal medicine decoction. Indian J. Dermatol. 56, 329-331.

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