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HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EFFECTS OF TATTOO INKS ON LYMPH NODES OF MALE HAMSTER

Abeer S. Abd Ali* and Gazwa D. Al-Nakeeb

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

Abstract

This research aimed to determine the immunohistochemical (IHC) and histological changes of tattoo inks (From original and fake companies) in the lymph nodes of hamsters. An adult male hamsters (n = 40) were randomly divided into eight groups. 6 groups injected with original ink (red, black and green) and fake inks (red, black and green) and 2 groups as a control. After sixty days, the hamsters were sacrificed and lymph nodes were isolated for immunohistochemical and histopathological studies. The results showed increased the Langerhans cells in the lymph nodes of animals tattooed with original inks comparison with the control and groups tattooed with fake inks. Also, histopathological changes included congestion of blood vessels, hypertrophy and hyperplasia for each of the capsule and blood vessel walls in most tattooed groups. Sections showed hyalinization, amyloid, giant cells, nuclei pyknosis and severe necrosis included both cortex and medulla. Severe lipomatosis, fatty changes and small lymphocytic lymphoma were seen. Also, the deposition of tattoo pigments was noticed in the axillary and inguinal nodes of all tattooed hamsters. All histopathological changes were more intense in the lymph nodes of animals tattooed with original inks than the lymph nodes tattooed with fake inks. Tattoo inks caused dangerous effects on all lymph nodes. Further research should be carried out to discover the immunohistochemical effects on B and T cells in the lymph nodes.

Key words: Tattoo inks, lymph nodes, hamsters, CD1a

Introduction

Tattooing is the process of inscribing the skin with an exogenous material (Inks and Pigments) (Kirby *et al.,* 2009). The word of tattoo originated from Tahitian word ta-tau which means to mark (Yashim *et al*, 2017). Tattoos are classified into different types including decorative, cosmetic, medical, temporary and traumatic (Sweeney, 2006; Caucanas *et al.,* 2011; Koljonen and Kluger, 2012). In the last years, tattooing practice becomes more popular and millions of people around the world have one or more tattoos. The precise composition of tattoo inks is very complex and tattoo inks are usually composed of different components involving binders, solvents, additives and pigments, the latter are either organic or inorganic (Kluger, 2008). During the tattooing, tattoo ink injects into the skin by needles of quickly vibrating machine, the needles form punctures and causing pierce the thin epidermis reach to the superficial dermis and maybe the middle dermis. Therefore, tattooing destroys the skin making superficial bleeding and pain. So, various side effects produce immediately or after tattooing, such as moderate swelling, itching, crusting and infectious disease (Klügl *et al*, 2010; Bäumler, 2015). Over time, some of the pigments are transferred to other organs in the body causing various complications (Kluger and Koljonen, 2012). This study aimed to explore the immunohistochemical and histopathological effects of different inks color (Original and Fake inks) on the lymph nodes of Syrian hamster males.

***Author for correspondence :** E-mail : abeersajid8@gmail.com

Material and method

Tattoo inks Collection

The tattoo inks utilized in Iraq are always imported, entire products that are made abroad. In this study, (6) colors of tattoo inks were used. (3) colors (Jet black, real red and green=(Grasshopper)) were collected from AL-Shorjah markets in Baghdad, that supply the tattoo centers in the cities of Locals (BioTouch and INTENZ- USA fake companies). And the other (3) colors (Lime Green, lining black and lipstick red were selected from tattoo centers in AL-Karada and AL-Rubaie Street in Baghdad

(Eternal Tattoo Supply-USA Original companies).

Experimental protocols and tattoo application

(40) Syrian hamsters were divided into (8) groups. Each group contains (5) hamsters in a separate cage. Before applying tattoo, animals were exposed to anesthesia (1:2 Ketamine: Xylazine), shaved the skin in the back area of each hamster and the shaved skin was sterilized to avoid microbial infection. With the help of a professional tattooist, tattoo inks were injected into the back skin (1cm in width and 4cm in length) for each hamster except control groups that tattooed without anything Fig. 1. This process was performed in sterilized conditions to avoid contamination and microbial infection.

Immunohistochemical and histopathological study

After sixty days, animals were sacrificed and lymph nodes were isolated for immunohistochemical and histopathological studies. Lymph nodes were fixated with a 10% concentration of formalin solution for (24) hours and immunohistochemical sections were prepared at 4 µm to detect the Langerhans cells by using the method of an avidin-biotin complex with the monoclonal antibody CD1a (Patho sito Leica). For histopathological study The lymph nodes were transmitted to a boun's solution as a fixative agent, then the paraffin blocks sectioned into 7µm and stained with routine hematoxylin and eosin according to (Suvarna *et al.,* 2013).

Results

The architecture of lymph nodes in control and tattooed groups consists of a capsule, cortex involved follicles and interfollicular areas which were less remarkable and contained a few Langerhans cells scattered in both cortex and medulla showed positive staining for CD1a marker Fig. 2-A and B and Fig. 3-A, B and C. While the Langerhans cells in the lymph nodes of animals tattooed with original inks were more massive and reflected by a more pronounced expression of anti-CD1a in comparison with the control and groups tattooed with fake inks Fig. 2-C-F. The histological alterations of lymph nodes represented by congestion of blood vessels, hypertrophy and hyperplasia for each of the capsule and

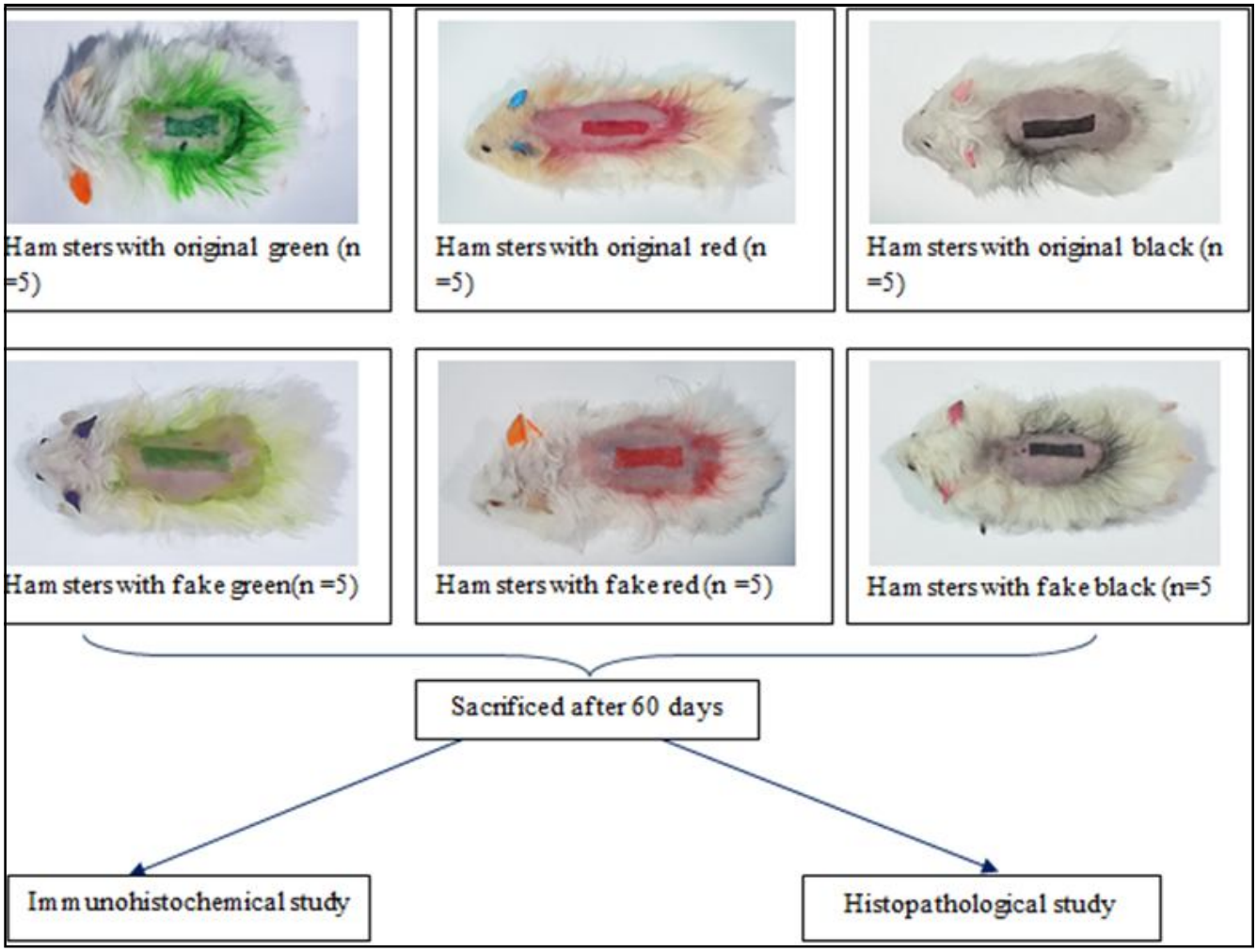

Fig. 1: Schematic diagram illustrates the study design.

blood vessel walls in most tattooed groups Fig. 3-D-F. Also, the sections showed hyalinization and amyloid Fig. 3-F, G, nuclei pyknosis in the lymph nodes of hamsters tattooed with original green ink Fig. 3-H and various degrees of necrosis included both cortex and medulla with atrophy of lymphocytes Fig. 3-G and Fig. 4-A. Severe lipomatosis, fatty changes and Metaplasia were seen in the lymph nodes of groups that were tattooed with original red and green inks and some animals tattooed with fake inks Fig. 4-B, C. Besides, axillary and inguinal nodes of animals tattooed with green and black original inks showed fibrosis and small lymphocytic lymphoma

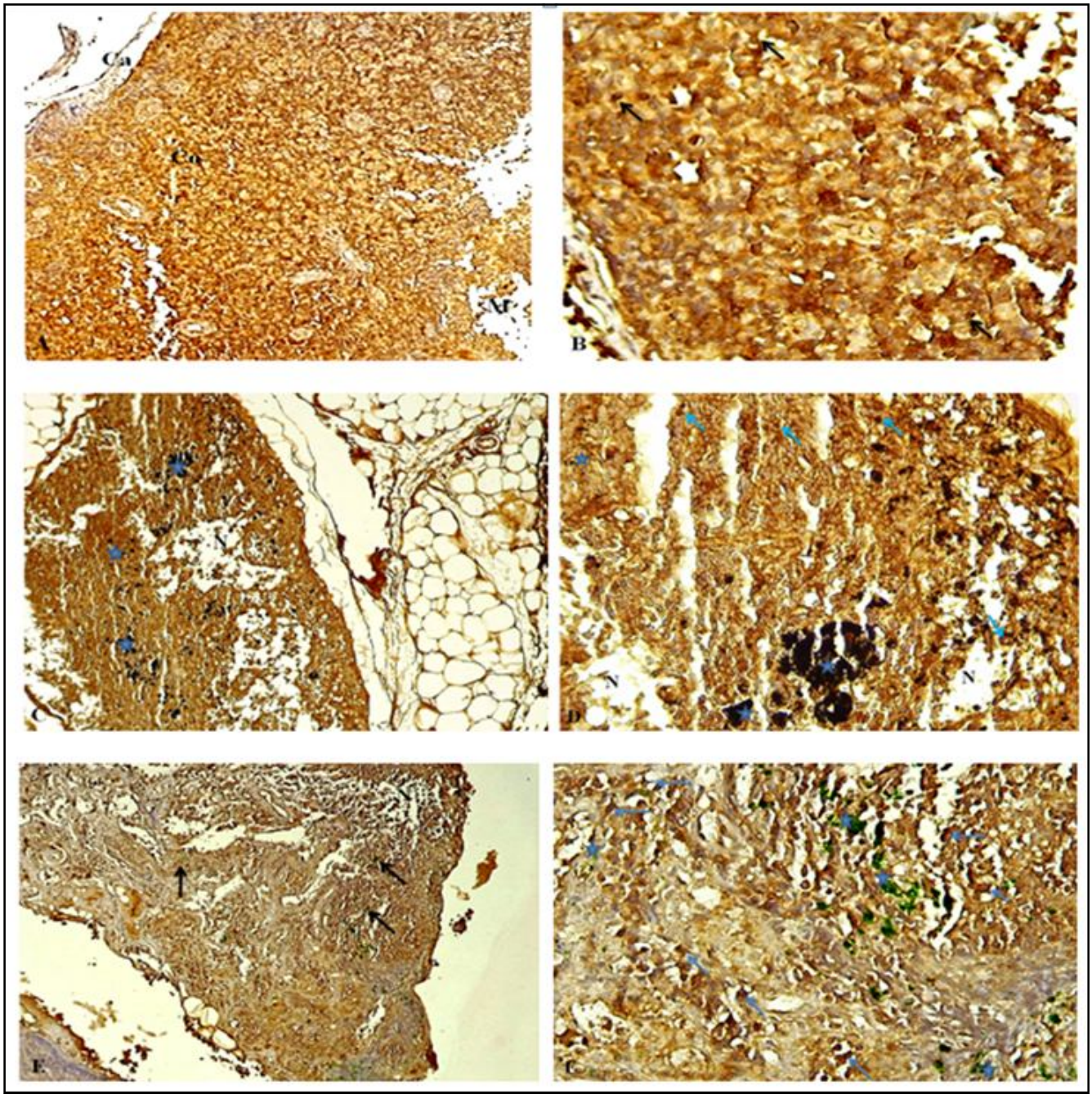

Fig. 2: Sections of lymph nodes of control group showing CD1a expression on A: 100x and B: 400x . Ca: Capsule, M:Medulla, Co: Cortex and (arrows): Langerhans cells; C and D: Sections of lymph nodes of hamster tattooed with fake black ink showing expression of anti-CD1a and tattoo pigments accumulation in an interfollicular and follicles areas. C: 100x and D: 400x. (stars): sites of pigments deposition and (arrows):Langerhans cells; E and F: Sections of lymph nodes of hamster tattooed with original green ink showing expression of anti-CD1a and tattoo pigments accumulation in an interfollicular and follicles areas . E: 100x and F: 400x. (stars)and (black arrows):sites of pigments deposition and (blue arrows): Langerhans cells.

(SLL) Fig. 4-D, E. As for the deposition of tattoo pigments, it was noticed in the axillary, accessory axillary and inguinal nodes of all tattooed hamsters Fig. 5. All histopathological changes mentioned above were more intense in the lymph nodes of animals tattooed with original inks than the lymph nodes tattooed with fake inks.

Discussion

The present study aimed to investigate the chronic effects of the tattoo inks from two sources (Original and fake companies) which are prevalent use in Iraqi tattoo centers on the lymph nodes of Syrian hamsters. The lymph nodes (Axillary, accessory axillary and inguinal nodes) of tattooed groups appeared tattoo pigment deposits in both cortex and medulla but the nodes of the animals tattooed with original inks showed more pigment deposits compared with the fake inks. These results stated by (Beavis *et al*., 2012; Jack *et al.*, 2005; Honegger *et*

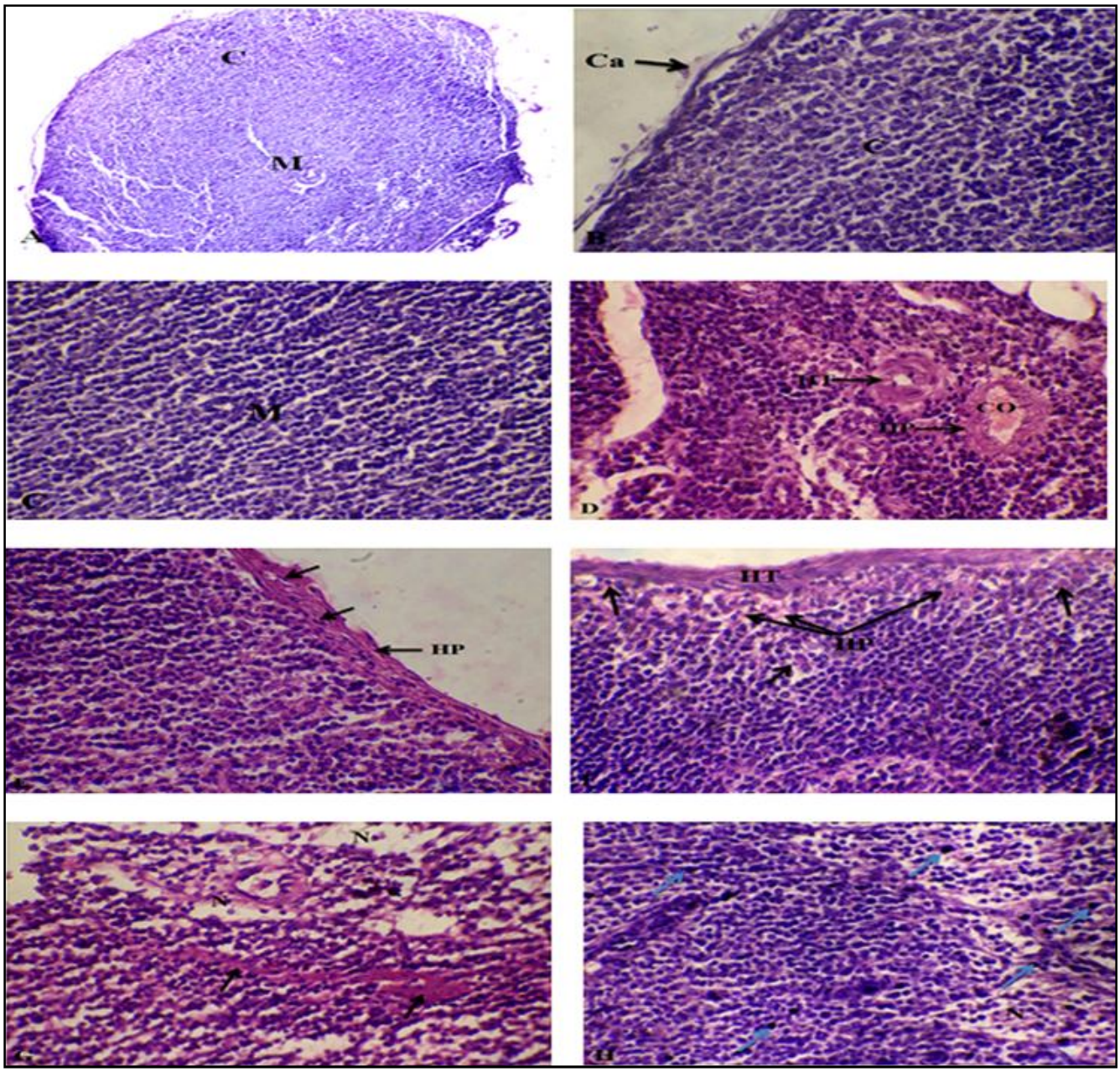

Fig. 3: Sections of lymph nodes of A-C: control group C (Cortex), M (Medulla), Ca (Capsule), (A):(100x) and (B,C) :(400x)D: Tattooed hamster illustrate congestion (Co) , hyperplasia and hypertrophy of blood vessel walls (HP & HT) (400x), E: Tattooed hamster illustrate Hyperplasia of capsule (HP) and fibroblasts (arrows) (400x),; F: Tattooed hamster illustrate Hyaline degeneration (arrows), Hypertrophy of capsule (HT), Hyperplasia of trabeculae accompanied by atrophy of lymphocytes(HP)(400x); G:Tattooed hamster illustrate Amyloid of sinusoid (arrows) and necrosis (N) (400x); H:Tattooed hamster illustrate pyknotic nuclei (arrows) (400x), (H&E).

al., 2004; Littonand Ghate, 2020; Gopee *et al.,* 2005) and attribute that the tattoo ink rests in the dermis either between bundles of collagen fibers or inside the fibroblasts (Kluger *et al.,* 2011). Macrophages in the skin

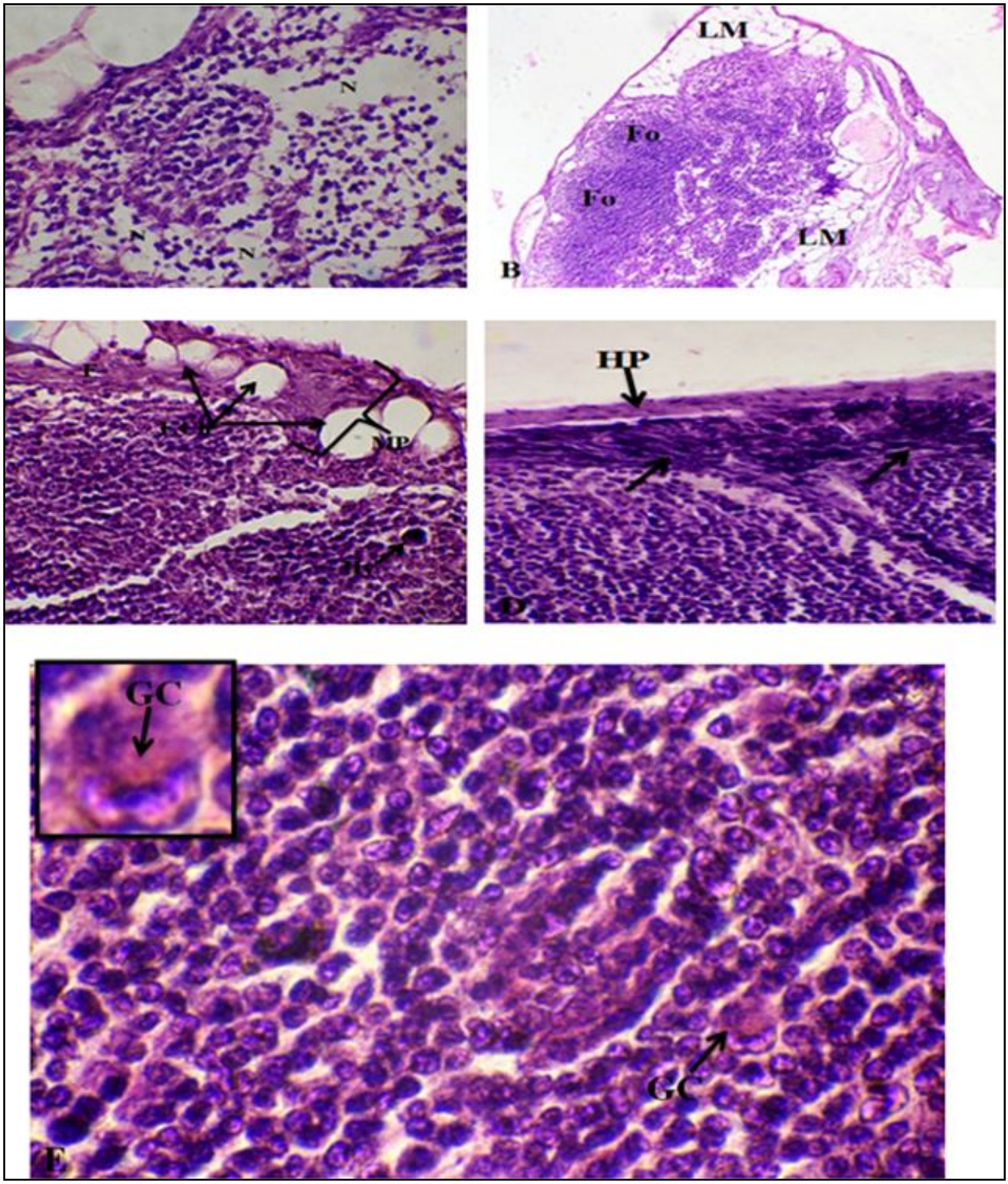

Fig. 4: Sections of lymph nodes of tattooed hamsters illustrate A: Severe necrosis (N) (400x),B: Lipomatosis (LM),Follicles (Fo), (400x). C: Metaplasia of capsule (MP), Fatty changes (F.Ch), Hyaline degeneration (Hy) (400x). D: Fibrosis (arrows), Hyperplasia (HP) (400x). ; E: Small Lymphocytic lymphoma, abnormal lymphocytes with clear cytoplasm, and giant cell(GC)(1000x), (H&E).

phagocytosed the pigments and migrate to the lymph nodes through the lymphatic vessels. In the lymph nodes, pigments may remain within the macrophages or deposit between the lymphocytes (Kluger *et al.,* 2008; Serup *et al.,* 2016). Pigments of most tattoo inks are mixing of numerous metallic ions usually iron, aluminum and titanium and if pigments exist in an adequate amount in axillary nodes, it may simulate calcification (Honegger *et al.,* 2004) or may simulate metastatic malignant melanoma (Jack *et al.,* 2005).

The tattoo pigment particles have a size range of 2-400 nm (Høgsberg *et al.,* 2011). So the nanoparticles of tattoo pigments can enter the human body compared with large particles therefore these particles enter the bloodstream and transfer around the body, taken by cells and tissues leading to oxidative stress and generate reactive oxygen species, cytokines production and cell death (Bocca *et al.,* 2017). Also, the nanoparticles are more likely of taken by mitochondria and nucleolus causing damage to the mitochondrial function and DNA (Revell, 2006). All the previous reasons, induce inflammatory responsescausing various histopathological alterations including blood vessel congestion. Perhaps caused by the effect of nanoparticles on the blood vessel walls leading

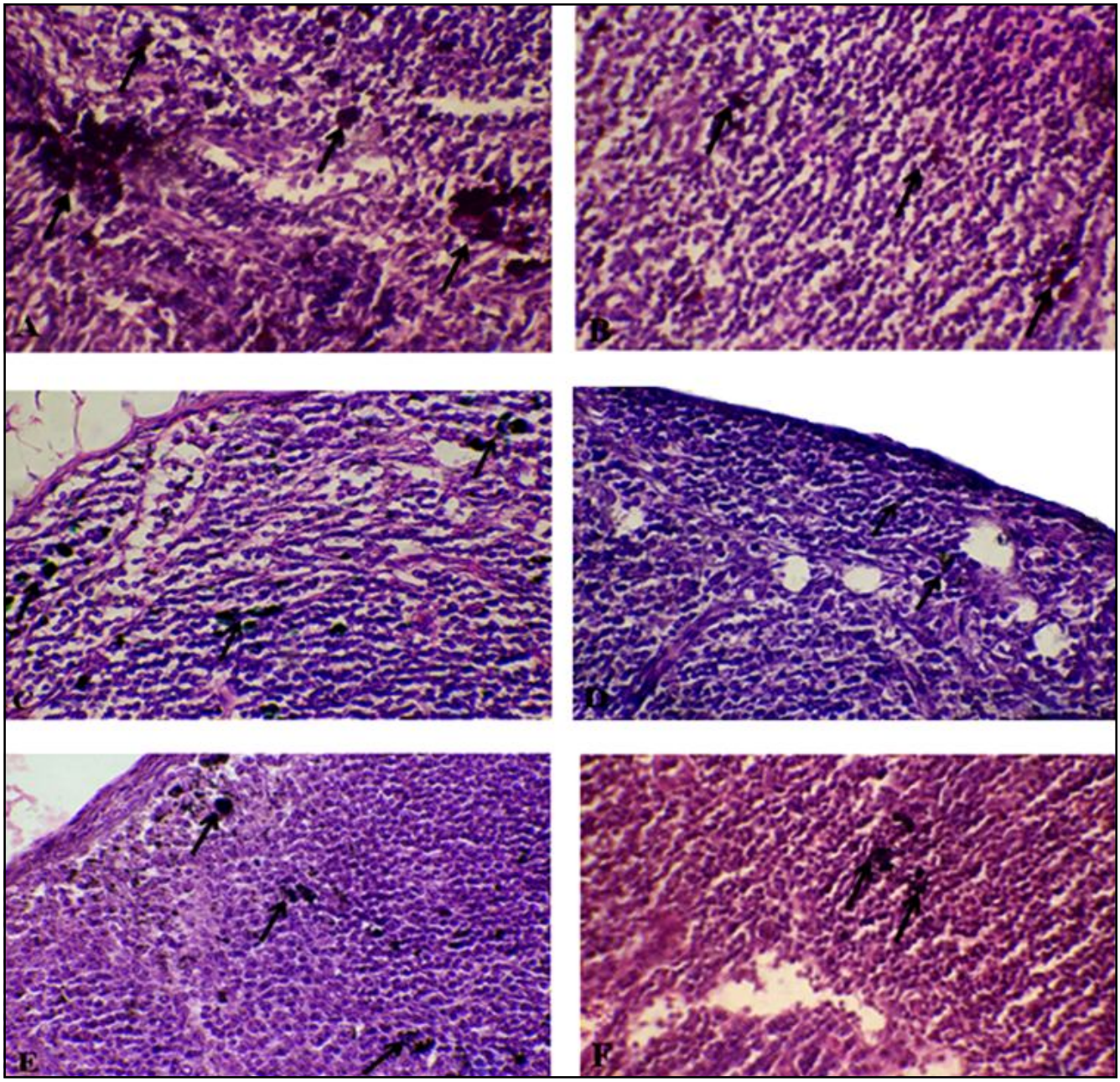

Fig. 5: Sections of lymph nodes of tattooed hamsters illustrate tattoo pigments deposition A, C and E: an original inks (Red, Green and Black), B, D and F: fake inks (Red, Green and Black),(400x), (H&E).

to dilating them and then resulting in the filling of the vessel with an abundant amount of blood (Robbinsand Kumar, 2007). Also, tattoo pigments caused deposition of proteins causing hyaline degeneration. And due to the inability of some lymphocytes in most lymph nodes of tattooed hamsters to adapt to the changes caused by the impact tattoo particles, causing their necrosis and consequently their death by the effect of the cytochrome C released from the mitochondria (Kumar *et al.,* 2007; Stevens *et al.,* 2009).

In this study, histopathological changes included fibrosis, was seen in most lymph nodes of tattooed groups. This may be explained by the effects of tattoo particles on the fibroblasts by inducing inflammatory reactions which results in the secretion of different cytokines such as IL-1 that activate fibroblasts and thus increased fibers synthesis (Solis *et al.,* 2002). On the other hand, the histological effects of tattoo nanoparticles were also hyperplasia for the connective tissue of the capsule and blood vessel walls, which is the behavior or adaptation of the tissue to resist the effect of nanoparticles leading to the thickening of the capsule and walls of the vessels (Kumar *et al.,* 2007). Also, tattoo pigments caused metaplasia of capsules by the accumulation of adipocytes within it and lipomatosis of lymph nodes produced by the accumulation of abnormal quantities of fats within the lymph nodes which may mimic lymphoma or other neoplasms.

Lastly, severe effects of tattoo pigments involved small lymphocytic lymphoma (SLL). It may be supposed that the inks contain a carcinogenic material involved heavy metals (Fritschi *et al.,* 2005) and PAHs (Regensburger *et al.,* 2010). These compounds may cause a chromosomal deletion and leading to loss of genes coding to micro-RNAs which act as a negative regulator for BCL2 and subsequently overexpression of BCL2 resulting in SLL (Jia *et al.,* 2008). As BCL2 gene represents an apoptosis inhibitor and participate in cell survival. The increase of necrosis, fibrous tissue, fat and amyloid in lymph nodes were excessive. These were considerably connected with a decrease in lymphocytes. Lymphocytes and other immune cells are necessary for a suitable lymph node function. Their partial loss might lead to insufficiency of lymphocytic fluid filtration and perform fast and enough immune response (Hadamitzky *et al.,* 2010). On the other hand, the histological changes were more intense in the lymph nodes of hamsters tattooed with original inks than the lymph nodes of hamsters tattooed with fake inks. This may be related to the differences in the composition of inks, as the original tattoo inks contain higher concentrations of heavy metals such as titanium, mercury, chromium, nickel and ferric oxide which are more likely to fixate the light color for a long time in comparison with the fake inks.

While IHC staining showed that the anti CD1a was more expression in the lymph nodes of tattooed hamsters compared with normal groups. Increase of Langerhans cells in lymph nodes may attribute to the fact that the inks may contain high concentrations of heavy metals and azo pigments, which are chemical sensitizers and penetrated the skin during the ink injection. These sensitizers covalently link with the epidermal proteins to form new antigens that are recognized by antigen-presenting cells that will encounter these antigens and migrate to the lymph nodes to expose the antigens to the T-lymphocytes and induce an immune response. Or, it is possible that during the tattooing, a microbial injury occurred and the Langerhans is considered the first defense line for pathogens in the skin. And during microbial facing, germline-encoded pathogen-recognition receptors distinguish molecular patterns on microbes, leading to the secretion of the pro-inflammatory cytokines and later promoted LCs mobilization to the lymph nodes causing increase LCs in lymph nodes (Egan and Jaffe, 2018).

Conclusion

The inks used in this study (from original and fake companies) cause severe effects on lymph nodes. So, further studies are necessary to investigate the physical and chemical compositions of inks and their acute and chronic effects on other organs.

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