

Histopathological Alterations in the Spleen Following Formalin-Induced Acute Dermatitis of Male Rats

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Abstract

Formalin is one of the liquid materials handled by the laboratory in particular and manufactured in general. It is also incorrectly disposed of and likely to be scattered to the main estuaries, which may significantly affect aquatic and wild animals. The primary goal of the study was to determine the changes in body tissues, particularly the spleen. The experiment involved taking five male rats for each group, injected with a saline solution under the skin of the rats, and considered this group to be the control group, which was called group (A). Group (B) was injected with a 2% diluted formalin solution under the skin for a week every 72 hours. The experiment on five other animals, with injections of the same substance and quantity under the skin, lasted for two weeks and was called group (C), comparing the results of the totals with some. The results of the tissue sections after routine staining (H and E) showed that the effect of formalin in group B was greater than in group C. After these totals were compared with the control group A, showed atrophy of the white and red pulp in the spleen. The atrophy of the white pulmonary lymphocytes includes loss of lymphocytes in T cells, and B cells. The study revealed that exposure to formalin causes significant alterations in spleen tissue the longer the exposure period.

Keywords: formalin; spleen; inflammatory.

Introduction

Many persons working in anatomy and embalming are exposed to formaldehyde, which is a relatively toxic material. (1) Formaldehyde is a highly reactive, colorless, smelly gas that dissolves in water and can irritate the skin and eyes. (2) Formalin has several medicinal applications, including embalming animals, preserving surgical specimens, and preparing specimens for anatomy. (3) When the body is exposed to foreign substances such as microorganisms and toxic substances, it triggers inflammation, to varying degrees. (4), Elimination of pathogenic stimuli and restoration of affected tissues are made possible through this immune response. (5) The skin is the first line of defense against pathogens and dangerous chemicals, if these agents cross the skin, innate immunity begins, which causes acute inflammation. (6) Typical signs of inflammation include redness, high temperature, swelling, and discomfort. (7) Due to the increasing advances in the field of medicine, the current understanding of inflammation has expanded greatly over the years and there are still some hidden issues. (8) Previous research has shown that exposing the body to external contaminants causes inflammation in both significant and secondary organs. (9-10-11) This was discovered in a study that looked at changes in tissue that occur after feeding mice with a type of toxin called Dioctyl sodium sulfosuccinate (DSS)

Poison (12), If amyloid infiltration and dead cells are detected in all lymphocytes in mouse spleen (13), however, a study confirmed that exposure to toxic substances such as formalin and other chemicals causes tissue pathological changes. This is observed in internal organs (liver, kidney, heart muscle, lung, stomach, and nerve tissue) of experimental mice, including congestion, degeneration, fibrosis, negation, necrosis, and discharge. (14) A large body of research has revealed that injecting animals with formalin enhances pain by stimulating neurons in the central nervous system. (15) On the other hand, inflammatory sites emit mediators that promote inflammation, including cytokines and chemokine's, they cause pain behavior by enhancing the sensitivity of peripheral neurons that detect pain, it causes inflammatory proteins, including cytokines (TNF-Alpha, IL-1) and other inflammatory agents, to be produced. (16) At the same time, they cause an increase in intracellular oxidation, which in turn causes alterations in superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) own unique set of triggers and control mechanisms, it is thought that inflammation, because of its vast and ubiquitous nature. (17) Exposure to high concentrations of formaldehyde during pregnancy is more likely to affect embryonic development and create heart defects. (18) The main objective of this study was to determine the changes that occur in spleen tissue due to exposure to formalin at different time periods.

Materials and Methods

Laboratory animal husbandry and laboratory

Laboratory animals (male) brought male Rats from the Iraqi Center for Research on Cancer and Medical genetics. The animals were bred at the Animal House of the Faculty of Education for Pure Sciences - Basra University, under an appropriate temperature of 25-20 degrees Celsius. They included 12 hours and 12 hours of darkness. fed on a leech of 30% flour, 20% milk, 30% bran, and 30 water, for the purpose of proliferating it and getting enough of it to complete the experiment, after which female Rats from the multiplication process were isolated) with close ages (four months) and recorded weights (190 – 220 g) of laboratory animals.

Subcutaneous injection method

Rodents were placed into three groups, each with five individuals. Five of them were from the negative control group (A), which received normal saline injections. Both groups (B and C) were injected with formalin at a concentration of 2% by 20 micrometers. If the injection period of group B is a week, group C lasts for two weeks, with the duration of the injection being 72 hours for both groups. (B) was dissected one week following the final injection of formalin. The second group (C) was dissected two weeks following the final injection of formalin. To assess the chemical's impact, the organs were extracted and compared to the negative control group. (19)

Investigation of Histopathological changes in tissue alterations in rat spleen

In this research study, the method Humason was adopted in preparing passages to study the pathological effects of formalin.(20) Animals were dissected, and organs were fixed in a beaker containing 10% formalin for 48 hours. Samples were dried in ethanol solutions, submerged in xylene, and immersed in paraffin wax. Tissue slices were flattened with paraffin strips and cut to 7 µm thickness. The seats were dried, wax removed, and run through ethyl alcohol for 5 minutes each. Canada balsam was used, and the cover was slipped, examined with a Leica imaging microscope, and photographed. The results provide valuable insights into animal anatomy.(21)

Results

The results of the tissue section of the spleen showed that the effect of formalin in group (B) was greater than in group (C) compared with group (A), the negative control. This was demonstrated through histological changes, which showed atrophy of the white and red pulp of the spleen. The atrophy of the white lymphocytes includes the loss of lymphocytes in T cells (lymphoma surrounding arteries) and B cells (experiments, microbial centers, and marginal zones), figures (1, 2, and 6) illustrate this. There is an abnormal and abundant proliferation of macrophages throughout the red and white pulp of the spleen, as seen in Figure 4. compared to Figure 3.

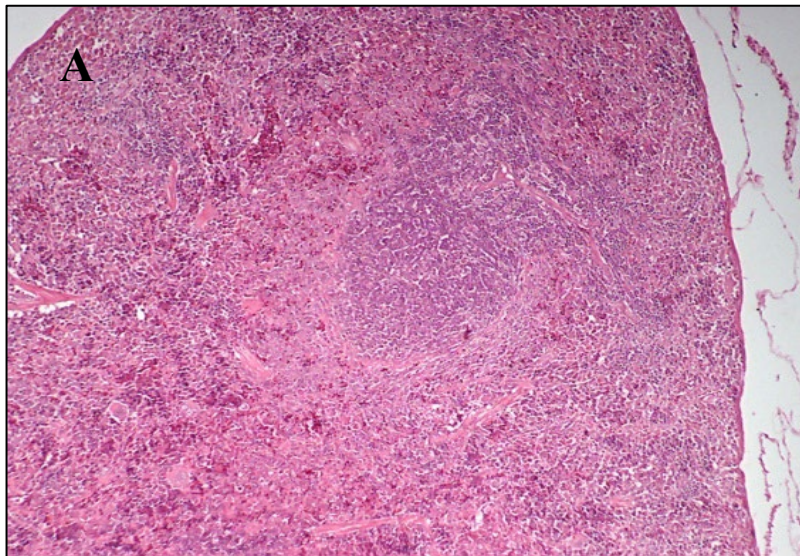


Figure (1) microphotography is a section of the spleen of group (A) mice, the red and white pulp with normal structure, 10x (H&E).

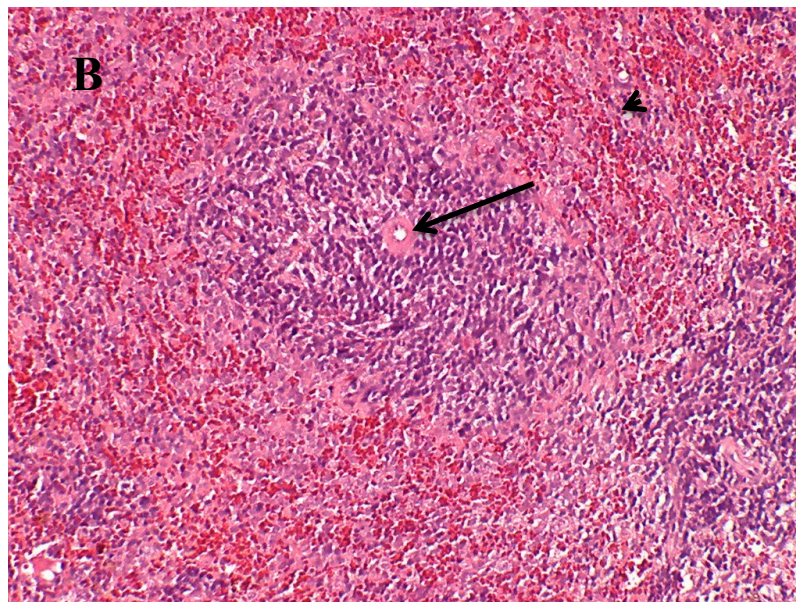


Figure (2) section of the spleen of group (B) mice. The white pulp lymphocytes are atrophic (arrows), and there is (arrowhead), thrombosis of the blood vessels 20x (H&E).

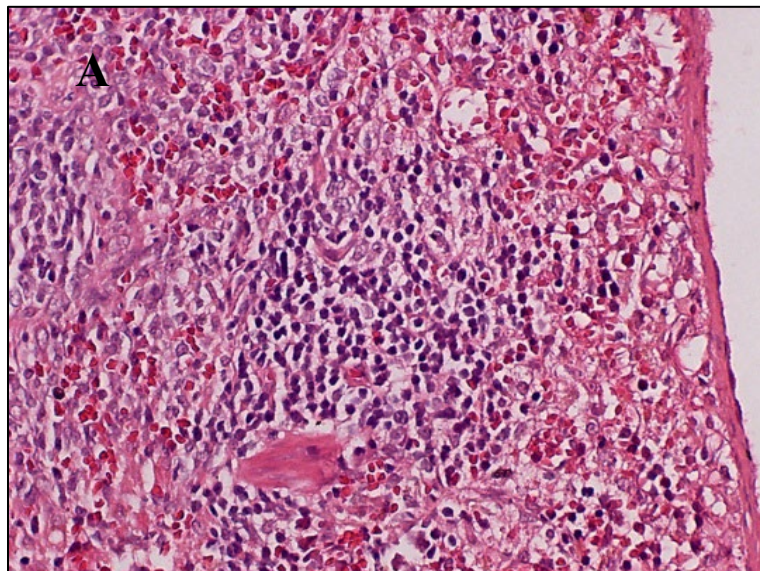


Figure (3) section of the spleen of group (A) rat , the red and white pulp with normal structure, 40x (H&E)

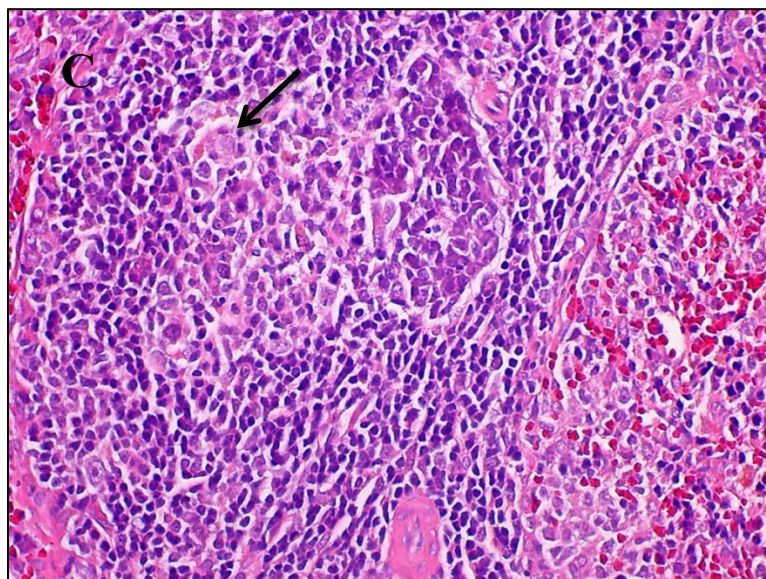


Figure (4) section of the spleen of group (C) rat, tangible body macrophages are scattered throughout the splenic white pulp (arrows) 40x (H&E).

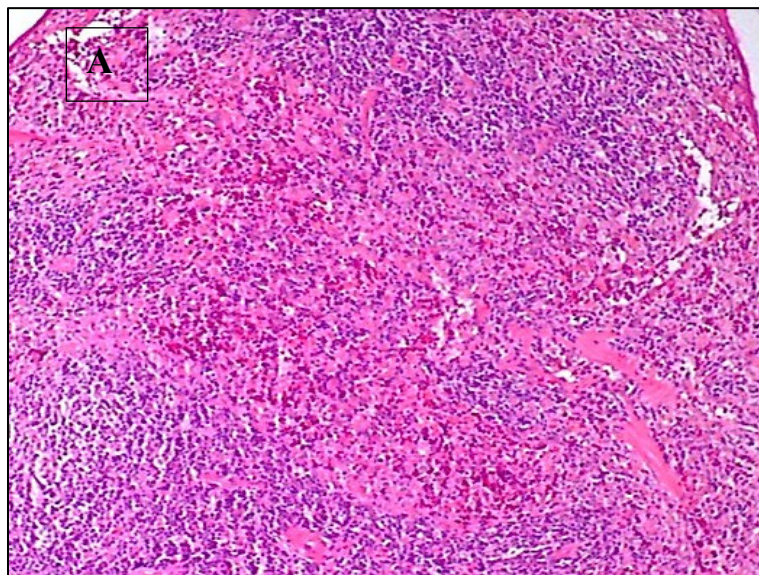


Figure (5) a section of the spleen of group (A) mice, the red and white pulp with normal structure, 20x (H&E)

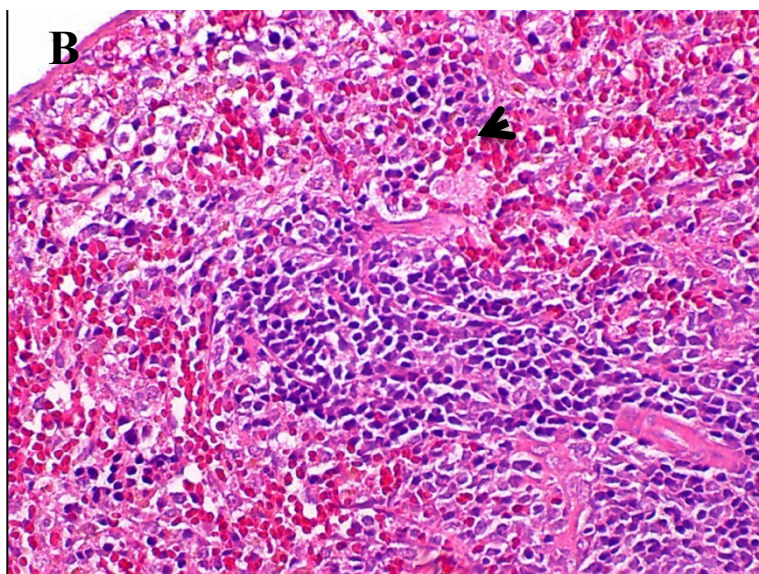


Figure (6) microphotography is a section of the spleen of group (B) mice, multiple variably sized blood-filled spaces lined by endothelium (head arrow) are present within the splenic parenchyma. 40x (H&E).

Discussion

During the current study, after exposing two groups of rats to the injection of a chemical substance directly under the skin, this induced inflammation within the body due to the entry of the foreign substance, which in turn led to the activation of biological stimuli such as the response signals released by the damaged tissue that led to its exposure to the chemical (Formalin) to cause damage or dysfunction. (22), a guest of the same area and surrounding tissue, which is consistent with the study. (23)

This research has shown that the exposure of the body to formalin through subcutaneous injections leads to some changes in the appearance of spleen tissue if textile images show some cellular decomposition that has led to the emergence of small empty spaces (Figure 2, 4, and 6), which attributed to the presence of necrosis in spleen cells that was the result of high oxidative voltage in the body and, in particular, the blood vessel mentioned. (24) in his study. Still, these changes were consistent with the research study on the precise description of necrosis (25).

The appearance of apoptosis in lymphocytes, which usually occurs naturally in germ centers in rodent spleen. However, it can increase when the animal is exposed to serious chemical or external injury. Here, an unprecedented or faster stimulation of programmed cellular death in lymphocytes is observed in the white core part and is on its way to cellular death. It can be traced back to the existence of a dysfunction of free radicals in tissue. Oral exposure to

formaldehyde has also been shown to cause DNA damage, apoptosis, and device injury. This is consistent with the study. (26)

The cause of the first group (B) spleen can also be returned more than the other group (C). As formalin is a toxic substance in high concentrations that triggers the release of response signals, the more the response is evidence of increased signals within the body, which in turn leads to damage to tissue and cells, and this happened with spleen tissue at the beginning of exposure to the chemical in the first week, This was consistent with the study, which explained the overproduction of inflammatory cytokines as a result of formalin exposure to tissue damage, changes in circulation, organ failure and eventual death. (27)

Conclusion

This research shows that chemical exposure induces alterations at the cellular level inside the body's organs. Formalin is an inflammatory agent that affects the spleen, which is intricately linked to both the lymphatic and circulatory systems.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

References

- 1-Sun, X., Yang, C., Zhang, W., Zheng, J., - Ou, J., & Ou, S. (2025). Toxicity of

formaldehyde, and its role in the formation of harmful and aromatic compounds during food processing. *Food Chemistry: X*, 102225.

<https://doi.org/10.1016/j.fochx.2025.102225>

2-Hagos, S., Gebeya, D., & Teklay, A. (2018). Effects of formalin toxicity among preclinical i medical students, Adigrat University, Ethiopia. *Cough*, 42, 43-8. DOI: 10.18483/ijSci.1479;

3-Pandey, C. K., Agarwal, A., Baronia, A., & Singh, N. (2000). Toxicity of ingested formalin and its management. *Human & experimental toxicology*, 19(6), 360-366. <https://doi.org/10.1191/096032700678815954>

4-Medzhitov, R. (2010). Inflammation 2010: new adventures of an old flame. *Cell*, 140(6), 771-776.

5-Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6), 7204. doi: 10.18632/oncotarget.23208

6-Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). *Molecular cell biology 4th* edition. National Center for Biotechnology Information, Bookshelf, 9.

7-Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, 454(7203), 428-435. DOI <https://doi.org/10.1038/nature07201>

8-Ahmed, A. U. (2011). An overview of inflammation: mechanism and

consequences. *Frontiers in Biology*, 6(4), 274-281. DOI

<https://doi.org/10.1007/s11515-011-1123-9>

9-Prata, J. C., Da Costa, J. P., Lopes, I., Duarte, A. C., & Rocha-Santos, T. (2020). Environmental exposure to microplastics: An overview on possible human health effects. *Science of the total environment*, 702, 134455.

<https://doi.org/10.1016/j.scitotenv.2019.134455>

10-Agarwal, K. C., Vinayak, V. K., Ganguly, N. K., Kumar, M., & Chhuttani, P. N. (1978). Ecological effects of production of biogas from human excreta on the enteric pathogens. *Indian Journal of Medical Research*, 1978, Vol. 67, 737-743

11-Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., ... & Ren, H. (2016). Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. *Environmental science & technology*, 50(7), 4054-4060. <https://doi.org/10.1021/acs.est.6b00183>

12-Case, M. T., Smith, J. K., & Nelson, R. A. (1977). Acute mouse and chronic dog toxicity studies of danthron, dioctyl sodium sulfosuccinate, poloxalkol and combinations. *Drug and chemical toxicology*, 1(1), 89-101. <https://doi.org/10.3109/01480547709034429>

13-Khaleefah, H. A., & Jasim, A. B. (2022). Experimental study on the effect of bft toxin isolated and purified from clinical isolates of Enterotoxigenic Bacteroides fragilis on the liver, spleen and intestine of

mice. *Basrah Journal of Veterinary Research*, 21(S1), 1-13.

14-Zainab, W. K., Salman, H. A., & Ebraheem, M. K. (2017). Histopathological changes that induced in the internal organs of white rat after exposure to diazinon. *Bas J Vet Res*, 16, 223-39.

15-Li, Y., Ou, J., Huang, C., Liu, F., Ou, S., & Zheng, J. (2023). Chemistry of formation and elimination of formaldehyde in foods. *Trends in Food Science & Technology*, 139, 104134.
<https://doi.org/10.1016/j.tifs.2023.104134>

16-Sekiguchi, K., Takehana, S., Shibuya, E., Matsuzawa, N., Hidaka, S., Kanai, Y., ... & Takeda, M. (2016). Resveratrol attenuates inflammation-induced hyperexcitability of trigeminal spinal nucleus caudalis neurons associated with hyperalgesia in rats. *Molecular Pain*, 12, <https://doi.org/10.1177/1744806916643082>

17-Rahimi, K., Zalaghi, M., Shehnizad, E. G., Salari, G., Baghdezfoli, F., & Ebrahimifar, A. (2023). The effects of alpha-pinene on inflammatory responses and oxidative stress in the formalin test. *Brain research bulletin*, 203, <https://doi.org/10.1016/j.brainresbull.2023.110774>

18-Zhang, Y., Yang, Y., He, X., Yang, P., Zong, T., Sun, P., ... & Jiang, Z. (2021). The cellular function and molecular mechanism of formaldehyde in cardiovascular disease and heart development. *Journal of cellular and molecular medicine*, 25(12), 5358-5371.
<https://doi.org/10.1111/jcmm.16602>

19-Martins, M. A., de Castro Bastos, L., & Tonussi, C. R. (2006). Formalin injection into knee joints of rats: pharmacologic characterization of a deep somatic

nociceptive model. *The journal of pain*, 7(2), 100-107.
<https://doi.org/10.1016/j.jpain.2005.09.002>

20-Humason, G.L., (1972). animal tissue techniques W.H. freeman and GO., san francisco. *Animal tissue techniques.*, 1962 .

21-Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. *In Manual of histologic staining methods of the Armed Forces Institute of Pathology* (pp. xii-258).
22-Lawrence T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor perspectives in biology*, 1(6), a001651.
<https://doi.org/10.1101/cshperspect.a001651>

23-Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6), doi: 10.18632/oncotarget.23208

24-Augenreich, M., Stickford, J., Stute, N., Koontz, L., Cope, J., Bennett, C., & Ratchford, S. M. (2020). Vascular dysfunction and oxidative stress caused by acute formaldehyde exposure in female adults. *American Journal of Physiology-Heart and Circulatory Physiology*, 319(6), H1369-H1379.
<https://doi.org/10.1152/ajpheart.00605.2020>

25-Auerbach, A., Summers, T. A., Zhang, B., & Aguilera, N. S. (2013). Splenic manifestations of chronic autoimmune disorder: a report of five cases with histiocytic necrotizing change in four cases. *Histopathology*, 63(1), 19-28.
<https://doi.org/10.1111/his.12143>

26-Arici, S., Karaman, S., Dogru, S., Cayli, S., Arici, A., Suren, M., ... & Kaya, Z. (2014). Central nervous system toxicity after acute oral formaldehyde exposure in

rabbits: *An experimental study. Human & experimental toxicology*, 33(11), 1141-1149.

<https://doi.org/10.1177/0960327113514098>

27-Czaja, A. J. (2014). Hepatic inflammation and progressive liver fibrosis in chronic liver disease. *World journal of gastroenterology: WJG*, 20(10), 2515. doi: 10.3748/wjg.v20.i10.2515

التغيرات المرضية النسيجية في الطحال بعد التهاب الجلد الحاد الناجم عن الفورمالين في ذكور الجرذان

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الخلاصة

الفورمالين هو أحد المواد السائلة التي يتعامل معها مختبرياً بشكل خاص وصناعياً بشكل عام. كما يتم التخلص منها بشكل غير صحيح ومن المرجح أنها تنتشر إلى صبات الأنهار الرئيسية بعد التخلص منها، مما قد يؤثر بشكل كبير على الحيوانات المائية والبرية. وكان الهدف الأساسي من الدراسة هو تحديد التغيرات في أنسجة الجسم وخاصة الطحال. وتضمنت التجربة أخذ خمسة فئران ذكور من كل مجموعة وحقتها بمحلول ملحي تحت جلد الفئران، واعتبرت هذه المجموعة بمثابة مجموعة التحكم والتي أطلق عليها المجموعة (أ). تم حقن المجموعة (ب) بمحلول الفورمالين المخفف بنسبة 2% تحت الجلد لمدة أسبوع كل 72 ساعة. واستمرت التجربة على خمسة حيوانات أخرى، بحقن نفس المادة والكمية تحت الجلد، لمدة أسبوعين، وأطلق عليها اسم المجموعة (ج)، وذلك بمقارنة نتائج الإجماليات مع بعضها. أظهرت نتائج مقاطع الأنسجة بعد التصبغ بصبغتي (H&E) أن تأثير الفورمالين في المجموعة (ب) كان أكثر منه في المجموعة (ج) وبعد مقارنة هذه المجاميع مع مجموعة السيطرة (أ)، أظهرت النتائج ضمور اللب الأبيض والأحمر في الطحال. كما شمل ضمور الخلايا الليمفاوية البيضاء و فقدان الخلايا الليمفاوية التائية والخلايا البائية. وكشفت الدراسة أن التعرض للفورمالين يسبب تغيرات كبيرة في أنسجة الطحال كلما طالت فترة التعرض.

الكلمات الرئيسية: فورمالين , طحال , التهاب.