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Research Article

HISTOCYTO-ARCHITECHTURAL CHANGES OF WISTER RATS SPLEEN EXPOSED TO PHOTOGRAPHIC FIXER EFFLUENT

PATRICK UGOCHUKWU AGBASI[1]*, ANTHONY CHUKWUKA UGWU[2], JEVAS EKEZIE[1], CHRISTIAN IKECHUKWU ANICHEBE[2]

[1]Department of Prosthetics and Orthotics, School of Health Technology, Federal University of Technology, Owerri, Nigeria. [2]Department of Radiography and Radiological Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra, Nigeria. Email: patagbasi@yahoo.com

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ABSRACT

Objective: We investigated the effect of subchronic low dose of radiographic fixer effluent on the cytoarchitecture of Wister rat spleen. The extensive use of fixer chemicals in radiographic/radiological laboratories and the attendant discharge in to the environment of the fixer effluent especially in the developing world necessitated this study.

Methods: A total of 25 Wister rats that have not been used for any experiment were divided into three groups namely Group 1 with 5 rats which served as control and received only water, Group 2 had 10 rats and received 200 mg/kg (8% LD_{50}) of fixer effluent, while Group 3 with 10 rats received 400 mg/kg (16% LD_{50}) of fixer effluent. The fixer effluents were orally administered daily for the 28 days study period. The rats were given free access to feeds and water. 2 weeks study: Midway, on the 14th day of the study period (short term), 5 Wister rats each from Groups 2 and 3 which received 200 mg/kg and 400 mg/kg administration of fixer effluent respectively were anaesthetized, sacrificed and their spleens were harvested fixed in 10% formalin saline solution awaiting further histological preparations for short-term investigation. 4 weeks study: On the 28th day of the study period (medium term), the rats in control group, and the remaining 5 rats each from Groups 2 and 3 were anesthetized and sacrificed while their spleens were collected fixed in 10% formalin saline solution for histological preparations and analysis.

Results: Short-term administration of (200 mg/kg) fixer effluent showed inflammatory cells in the red and white pulp. The medium term administration of (200 mg/kg) fixer effluent showed infiltration of inflammatory cells in the red and white pulp and equally in the venous sinuses between the sinusoids and the parenchyma of the red pulp. Furthermore, necrosis was recorded in the red pulp parenchyma. Short-term administration of 400 mg/kg fixer effluent to the rats showed slightly enlarged red and white pulp with inflammation and moderate necrosis of parenchyma of the red pulp. There was also distortion in the germinal center and trabecular. Medium term administration of 400 mg/kg fixer effluent to the rats caused moderately enlarged red and white pulp with severe necrosis and lymphocytic infiltrations in the red pulp parenchyma and also infiltrations in the venous sinuses between the sinusoids and parenchyma of the red pulp.

Conclusion: The fixer effluent caused deleterious effects to the cellular structure of the rat spleen.

Keywords: Fixer effluent, Wister rats, Subchronic, Sinusoids, Inflammation, Lymphocytic infiltration.

INTRODUCTION

Radiographic X-ray film/photographic fixers are chemicals used in the final step of radiographic film/paper processing. It is applied on the photographic fixers or X-ray films/papers after chemical developers were applied. The radiographic fixers primarily neutralizes any developer remaining on the film, removes any underdeveloped silver halides which is a component of the film, leaving behind reduced metallic silver that forms the image which is also stabilized by the fixer [1].

Radiological fixers contain chemicals such as ammonium thiocyanate and boric anhydride. Ammonium thiocyanate reacts with undeveloped silver halide in the film to form silver thiocyanate. After bathing/immersion of the X-ray or photographic films/paper in the fixer container, the washout is called fixer effluent and the major chemical content of the effluent is a mixture of mainly silver thiocyanate and the boric compounds. Several films/papers are usually washed in the fixer container before discarding thereby increasing tremendously, the silver content of the washout. In many parts of third world countries, these effluents are discharged into the environment which contaminates food chain and sources of drinking waters both for animals and humans.

Spleen is a lymphoid organ found at the upper quadrant of the abdomen just below the rib cage in humans and other mammals. The spleen's roles such as boasting immunity and blood purifier by filtering of damaged and old red blood cell (RBC) from circulation defines its importance. The spleen produces the monocytes and lymphocyte that are important in phagocytosis and immunological response and defense in infection. The spleen stores blood and degrades the filtered damaged and old RBC into constituent amino acids, heme portion metabolized to bilirubin in the liver while iron is stored for reuse [2].

The spleen synthesizes antibodies in the white pulp area and filters out antibody-coated bacteria and blood cells from the systemic circulation. The red pulp also produces about half of the body's monocytes which are promoters of tissue healing and repairs [3,4].

In many parts of Africa and other third world countries due to nonexistent policy, poor regulation implementation were it exists, effluents from hospitals and photo/film laboratories including industrial wastes are discharged into the environment without treatment which endangers our food chain. Therefore, this study seeks to assess the toxicological effect of radiographic fixer effluents on the cytoarchitecture of Wister rat spleen.

METHODS

Study design

Acute toxicity study

An empty evaporating dish was weighed and recorded. Then 400 ml of the fixer effluent was placed in the evaporating dish and allowed to

evaporate by heating in a chromatograph oven. Three other volumes of 400 ml were also evaporated to get enough solute. At the end of the evaporation procedure, the solute powder was weighed and reconstituted with water to form our stock of fixer effluent solution. The LD_{50} was then determined to be 2500 mg/kg using the method described by Lorke [5].

Animals

This subchronic toxicity study was done with 16 (25) Wister rats which was bought from animal house of Faculty of Pharmacy Nnamdi Azikiwe University, Awka Campus, Nigeria and was allowed to acclimatize in our facility at College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus Nigeria for 2 weeks before the study was commenced.

Ethical considerations/approval

The research work was approved by the Department of Radiography review board of our School and Ethical Approval received from Faculty of Health Sciences of Nnamdi Azikiwe University, Awka, Nigeria. The guiding principles in the care and use of animals for experiment were employed: Animals were lawfully acquired, properly housed, fed and their environment kept in a sanitary condition, appropriate anesthesia was given to eliminate sensibility to pain before sacrificing them.

Study protocol

A total of 16 (25) Wister rats that have not been used for any experiment which had been earlier acclimatized in our laboratory for 2 weeks were used for the study. The animals were placed under 12 hrs light/12 hrs darkness cycle. They were divided into 3 groups namely Group 1 with 5 rats which served as control and received only water, Group 2 had 10 rats and received 200 mg/kg (8% LD_{50}) of fixer effluent, while Group 3 with 10 rats received 400 mg/kg (16% LD_{50}) of fixer effluent. The fixer effluents were orally administered daily for the 28-day study period. The rats were given free access to feeds and water.

2 weeks study

5 Wister rats each from Groups 2 and 3 which received low dose (200 mg/kg) 8% of LD_{50} and high dose (400 mg/kg) 16% of LD_{50} of fixer effluent orally respectively for 2 weeks were sacrificed on the 14^{th} day, i.e., 24 hrs after the last dose of administration of fixer. Their spleens were harvested for short-term investigation. The animals were anesthetized with diethyl ether for 2, minutes before they were sacrificed. The spleen was extracted and fixed in 10% formalin saline solution.

4 weeks study

On the 28^{th} day, the remaining 5 rats each from Groups 2 and 3 and the control group were also sacrificed on the same day following the same protocol as in the 2 weeks study. The spleen from the rats was harvested and fixed in 10% formalin saline solution.

Tissue preparation for histological examination

After thorough fixation in 10% formal saline solution for 24 hrs the Wister rat tissues were cut longitudinally into smaller pieces, respectively, before putting them in an automatic tissue processor for serial chemical treatment and preparations. After the tissues were processed and impregnated with paraffin wax, they were cut into very thin sections using microtome machine. The thin sections were collected on glass slides and then stained with hematoxylin and eosin technique. The stained slides were examined by our histopathologists with a light microscope attached with a digital camera which transferred the images to our personal computer.

RESULTS

The control spleen sample as shown in the histological slide of Fig. 1 shows intact cellular architecture of white pulp, red Pulp which make up the bulk of the spleen and the thin but dense fibro-elastic outer part. Short-term (2 weeks) administration of 200 mg/kg fixer effluent to the rats presented moderate pathological changes in the spleen with inflammatory cells in the white pulp, red pulp, and intra trabecular as seen in Fig. 2. Furthermore, medium term (4 weeks) administration of 200 mg/kg fixer effluent as shown in Fig. 3 caused infiltration of inflammatory cells in the white and red pulp. There was necrosis in the red pulp parenchyma area and also infiltrations in the venous sinuses between the sinusoids and parenchyma of the red pulp.

Short-term administration of 400 mg/kg fixer effluent to the rats as presented in slide Fig. 4 showed slight enlargement of red and white pulp, necrosis of parenchyma of the red pulp, inflammation of red pulp, distortion in the germinal center with distorted trabecular.

Medium term (4 weeks) administration of 400 mg/kg fixer effluent to the rats as shown in Fig. 5 caused moderately enlarged white pulp and severe necrosis with lymphocytic infiltrations in the red pulp parenchyma and also infiltrations in the venous sinuses between the sinusoids and parenchyma of the red pulp.

DISCUSSION

The red pulp makes up the largest part of the spleen and receives the arterial blood supply with venous sinuses which can be found throughout the red pulp. The red pulp receives the arterial blood first and this exposes it more to damage and harmful effect of chemical toxicants than the white pulp.

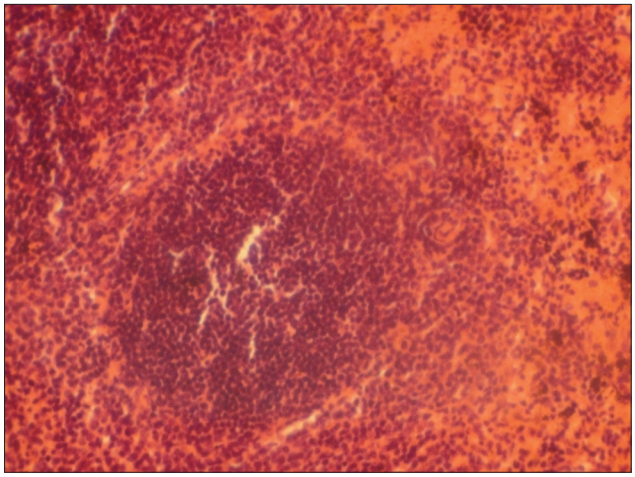

Fig. 1: Photomicrograph of the spleen of control rats that were administered distilled water orally for 28 days

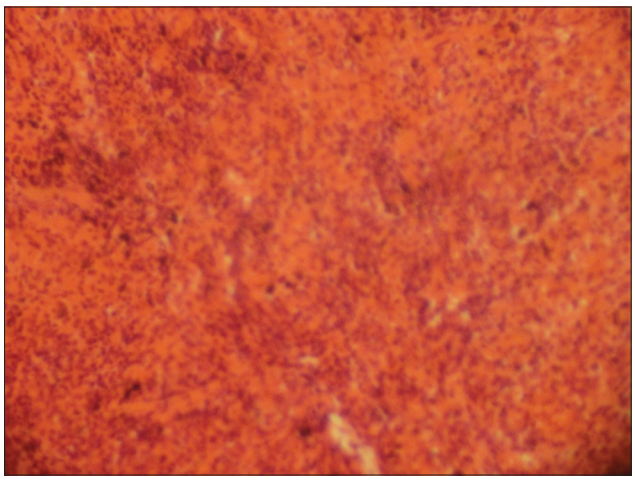

Fig. 2: The photomicrograph of the spleen of Wister rats (×400) of low dose (200 mg/kg) fixer effluent (short-term oral administration). The effect shows infiltration of inflammatory cells in the red and white pulp

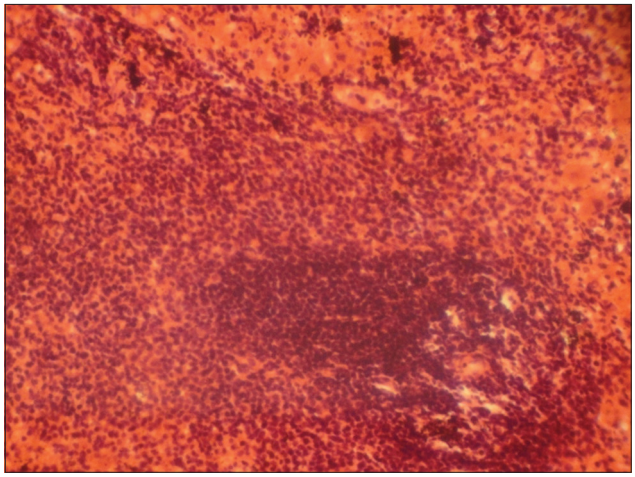

Fig. 3: The photomicrograph of the spleen of Wister rats (×400) of low dose (200 mg/kg) fixer effluent (medium term oral administration). The effect shows infiltration of inflammatory cells in the red and white pulp with moderate necrosis in the red pulp parenchyma area

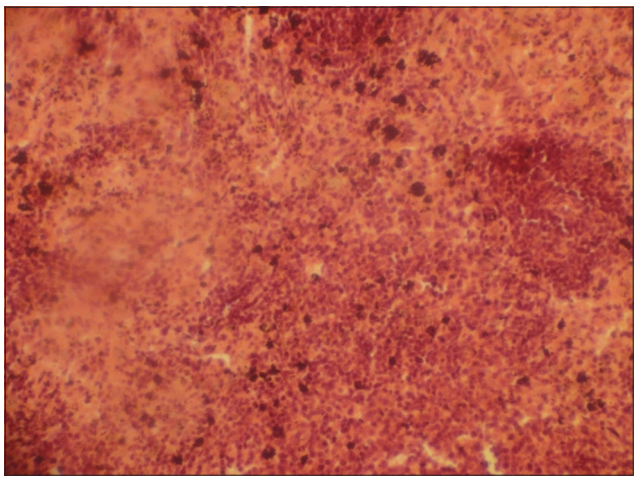

Fig. 4: The photomicrograph of the spleen of Wister rats (×400) of high dose (400 mg/kg) fixer effluent (short-term oral administration). The effect shows slight enlargement of red and white, infiltration of inflammatory cells in the red and white pulp with necrosis in the red pulp parenchyma area

The two functional zones of the spleen are the hematogenous red pulp and the lymphoid white pulp. Compounds inducing lymphocytes toxicity may cause necrosis of the white pulp. Necrosis in the splenic white pulp is typically characterized by apoptosis of lymphocytes. Spleen toxicity induced by chemicals has been reported. The administration of 1,2-dimethylhydrazine to rats caused deleterious effects to the spleen [6].

In the present work, the possible effect of fixer effluent on the spleen of a Wister rat has been assessed. Our study showed that short-term administration of low dose (200 mg/kg) fixer effluent caused inflammation of splenic cells in the red and white pulp. Similarly, sodium thiosulfate a component of fixer was used to determine the calcinosis in a rat model. It was observed that there were inflammatory conditions on the splenic cells in the red and white pulp [7], in our medium term administration of low dose (200 mg/kg) fixer showed inflammatory cells in the red and white pulp with attendant necrosis of the red pulp area. This is similar to a previous study by Gard and Abass [8] which shows that administration of clonazepam and alprazolam on albino rat causes depletion of lymphocytes from the spleens, lymph node, and inflammatory cells infiltrations.

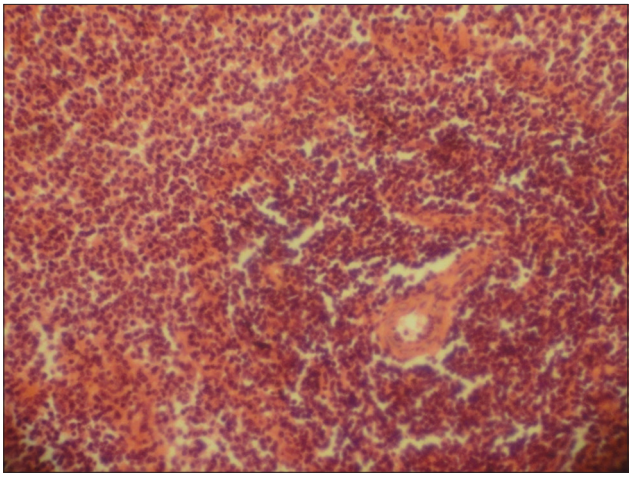

Fig. 5: The photomicrograph of the spleen of Wister rats (×400) of high dose (400 mg/kg) fixer effluent (medium term oral administration). The effect shows moderate enlargement of red and white pulp parenchyma, and also infiltration of inflammatory cells in the red and white pulp with severe necrosis in the red pulp parenchyma area

Short-term administration of high dose (400 mg/kg) fixer effluent caused enlargement of red and white pulp (hypertrophy), necrosis of the parenchyma of the red pulp, inflammation of the red pulp, and distortion of the germinal center. This is similar to one of the findings in a previous work on the morphology of the spleen in adult albino rats after whole body exposure to low level of toluene which led to hypertrophy of the white pulp of the spleen [9].

Medium term administration of high dose (400 mg/kg) showed slightly enlarged white pulp and lymphatic infiltrations in the red pulp, necrosis in red pulp parenchyma, infiltrations in the venous sinuses between the sinusoids, and parenchyma of the red pulp. A previous study on paraquat-induced toxicity in spleen of albino mice showed infiltrations of lymphocytes and neutrophils in the parenchyma of spleen [10]. Furthermore, clonazepam and alprazolam were equally reported to cause to spleen, severe necrosis in the red pulp parenchyma and also infiltrations in the venous sinuses between the sinusoids and the parenchyma of the red pulp [8]. Silver is the major content of fixer effluent [11] and silver have been shown to cause toxicological effects in other organs. According to Mansee *et al.* [12], silver nanoparticles caused depletion of germinal cell necrosis, fibrous leydig cells, and vacuolated sertoli cells.

Boric acid (a component of fixer) disrupted the normal functions of the spleen which is an organ important in filtering and storing blood [13]. Although boric acid has been reported to protect the lungs and liver cells from carcinogenic effects of aflatoxin B (1) [14]. According to WHO [15] report, toxicological effects on the spleen have been observed in animals chronically exposed to hydrazine by inhalation causing atrophy of the spleen.

Inflammatory reactions of the spleen occur in the context of two pathophysiological settings. First, lymphoid hyperplasia of the spleen can be the result of principally, physiological production of immune effector cells due to viral infections, autoimmune diseases, and acquired or inherited immunodeficiencies. Second, the spleen itself may be the target of a pathological inflammatory reaction of exogenous chemical introduced into the body. Fixer administered at high dose in 4 weeks shows inflammatory cells in the white pulp and inflammatory cells in the red pulp with severe necrosis in red pulp parenchyma and also infiltrations in the venous sinuses between the sinusoids and parenchyma of the red pulp. The proposed mechanism for action of fixer may be from one of its constituent (acetic acid and/or ammonium thiosulfate), but it's mechanism of action is not well understood. The

mechanism of action could be as a result of generation of free radicals which eventually caused tissue damage and also lipid peroxidation of the cell membrane of the spleen. Lipid peroxidation and generation of free radicals has been shown to be one of the sources of toxic effect of chemicals to tissues [16]. Sayed *et al.* [17] have demonstrated that *Phyllanthus emblica* leaf extract protected the spleen, kidney, and liver of mice from sodium arsenite-induced toxicity. There is a need for extensive research to assess the possibility of using plant extract/ traditional remedies to abolish the toxic effect of fixer effluent and other deleterious chemical compounds to the body organs especially the spleen.

CONCLUSION

Administration of fixer effluent at the doses of 200 mg/kg and 400 mg/kg induced inflammation of splenic cells, tissue necrosis, and observable architectural changes in the spleen.

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