



Original Research Article

# Hepatotoxicity of radiographic fixer effluent on wistar rats

Received 29 April, 2019

Revised 2 June, 2019

Accepted 8 June, 2019

Published 22 July, 2019

**Anthony Chukwuka Ugwu<sup>1</sup>,  
Nkiru Elvina Ignatius<sup>2</sup>,  
Michael Promise Ogolodom<sup>\*3</sup>,  
Beatrice Ukamaka Maduka<sup>4</sup>,  
Robert Oziegbe Akhigbe<sup>5</sup>,  
Awajimijan Nathaniel Mbaba<sup>6</sup> and  
Oluwafemi Olumide Egbeyemi<sup>1</sup>**

<sup>1</sup>Radiography and Radiological Sciences  
Department, Faculty of Health Sciences and  
Technology, Nnamdi Azikiwe University  
Anambra State, Nigeria.

<sup>2</sup>Afe Babalola University Multi-System  
Hospital Ado-Ekiti.

<sup>3</sup>Rivers State Hospital Management Board Port  
Harcourt, Rivers State Nigeria

<sup>4</sup>Department of Medical Radiography and  
Radiological Sciences, University of Nigeria  
Enugu Campus, Nigeria.

<sup>5</sup>Rovina Medical Diagnostic Services, Lagos  
State Nigeria

<sup>6</sup>Department of Radiology, Rivers State  
University Teaching Hospital, Port Harcourt  
Rivers State, Nigeria

\*Corresponding Author Email:  
[mpos2007@yahoo.com](mailto:mpos2007@yahoo.com)

Tel.:+234803997393

This study was designed to evaluate the hepatotoxicity of radiographic fixer effluent on Wistar rats. Fourteen Wistar rats of weights 140-220g were divided into three groups; the control group (I) and experimental groups (II and III). The control group was further divided into groups of two rats each and administered 1ml of distilled water daily orally for 14 and 28 days respectively, each of the experimental groups II and III were further sub-divided into two groups of two and three rats each respectively (i.e. group IIA, 2 rats, group IIB 3 rats and group IIIA 2 rats and group IIIB 3 rats), and were orally administered with 200mg/kg and 400mg/kg of fixer effluents daily for 14 days and 28 days respectively. Histopathological result showed normal liver tissues in the control group; liver tissue with hypertrophied (distended) central vein in groups II A and II B; normal hepatocytes, interstitial tissues and distended central vein with mild infiltrate of edematous fluid with long term experimental group; liver tissue enlarged central vein with indication of edematous infiltrate accumulation within the vein with short term experimental group. The present study showed that long and short-term oral administration of low dose fixer effluent, as well as short-term administration of high dose of fixer caused changes in the histological patterns of a healthy Wistar rats' liver.

**Key words:** Effluent, fixer, hepatotoxicity, liver

## INTRODUCTION

The liver is the second largest organ in the body that is roughly triangular, and is located beneath the diaphragm at the right upper quadrant of the abdomen (Guyton and Hall, 2005; Thomas and Leesson, 1965). It account for about 1.5kg of the total body weight in average adult human (Guyton and Hall, 2005) and performs functions such as filtration and storage of blood, metabolism of carbohydrates, fats, proteins, hormones and foreign chemicals, formation of bile, storage of vitamins and iron and formation of coagulation factors (Guyton and Hall, 2005). The liver is a special organ, in which its functions interrelate with one another. This is so because any evident abnormalities of the liver, which could result from chemical

contamination of food equally affects the liver functions simultaneously (Guyton and Hall, 2005).

The liver plays vital role in transforming and clearing chemical and is susceptible to the toxicity from these substances. Hepatotoxins are chemicals that cause liver injury and these includes; 2,4,5 Trichlorophenoxy, acetic acid may damage the liver (Bowen, 2007). Exposure to Dichloroacetic acid may affect the liver and kidneys. Dichloroacetate (DCA) caused nerve and liver damage, as well as some other side effects (Barkel and Hazelwood, 2005).

Histologically, the liver section examined under low power microscope shows that liver is composed of masses of

epithelial, parenchymal cells arranged in anastomosing and branching plates that form a three dimensional lattice (Thomas and Leesson, 1965; Burkell and Low, 1996). Exposure of the liver to any inflammatory agents could alter both the physiology and the histological patterns of the liver.

The production of radiographic images involves a chemical process known as fixation. Fixation is the process whereby unexposed and undeveloped silver halide (AgX) and silver bromide are removed from the emulsion layer of the photographic film material, and the hardening of gelatin component of the film emulsion (Ogolodom, 2015; Mosby, 2009; Gunn, 1994). This process involves the use of chemical substance called fixer, which comprises of ammonium thiosulphate, sodium thiosulphate compounds and other additives which are chosen based on their fast clearing time (Ogolodom, 2015; Gunn, 1994; Cullinan et al., 1992; Tseke and Chow, 2000; Warren, 2001).

Actually, plain (unused) fixer is non-hazardous to human. Nevertheless, the more repeatedly a fixing bath is utilized, the more silver halide from the film is deposited in it (Ugwu et al., 2017). The products of the reaction comprising of silver become extra complex, meaning that the silver molecules becomes larger and larger as the fixing bath continues through its working life until it reaches exhaustion (Ugwu et al., 2017). The exhausted fixer effluent is then discarded. The exhausted fixer effluent that is produced is deemed a hazardous chemical that can cause threat to public health or the environment (Adal and Wiener, 2015).

The environmental protection agency laws has enumerated the poisonous nature of silver metal and cautioned that, if silver is discharged along with other liquid into the environment, it can cause environmental pollution and harmful effect to human directly or indirectly through plants or animals, hence the need for silver recovery (Ogolodom, 2015; Gunn, 1994; Grigolletto, 2008). In radiography, the major source of silver is the used fixer, because it contains high silver content. Used fixer, which contain silver concentration of 5.0 parts per million (ppm) or more are usually hazardous since they depict the features of the toxicity (Harper, 2003; Wynmer, 2000).

Radiographers usually discharge radiographic fixer effluents into the environment without adequate knowledge of the environmental impacts of this effluent. There is scanty research work on the potential toxicological effect of fixer effluent on the liver in our locality. The result of this study would be beneficial to the radiographers, radiologists, the environmental scientists and the public as we aimed at evaluating the histopathological changes in the liver tissues of Wistar rats after short and long terms exposures to a fixer effluent.

## MATERIALS AND METHODS

### Animals

Fourteen apparently healthy Wistar albino rats weighing

140-220g were used in this study. They were housed in the animal house of the Department of Human Anatomy, Nnamdi Azikiwe University, Nnewi Campus, under standard conditions (29±2°C temperature, 40-55% humidity, good ventilation) and have free access to water and diet (normal rat chow). They were acclimatized for two weeks before the onset of the experiment.

### Test Chemical

The original commercially prepared fixer (a chemical used in processing photographic or x-ray films) was procured from Begood Manufacturing Company Ltd, China. The major components of the fixer are sodium thiosulphate and ammonium thiosulphate. The effluent, the liquid waste substance produced from radiographic processing encompasses of high silver-concentrated solution, sodium sulphite, sodium thiosulphate, acetic acid, boric acid and low PH. The lethal dose (LD50) concentration of the fixer effluent was calculated as 2450 mg/kg body weight using the formula:  $LD50 = \sqrt{a \times b}$  (where : a= the lowest dose that brought death i.e. 3000 and b= the highest dose that did not kill i.e. 2000). The lethal dose test of the fixer effluent was conducted at the Faculty of Pharmacy and Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus according to the approach adopted by Lorke (1983). The concentrations of the fixer effluent used for the experiment were sub-lethal dose of 200mg/kg (lower dose) and 400mg/kg (high dose) of body weight.

### Experimental Design

The animals were randomly divided into one control group (Group I) and two experimental groups (Group II and Group III). Group I consisted of four rats, group II and III consisted of five rats each. Group I was further divided into two groups of two rats each (i.e Groups IA and IB). These Wistar rats were given 1ml of distill water for 14 and 28 days respectively. The experimental groups (Groups II and III) received 200mg/kg and 400mg/kg of fixer effluent respectively. The experimental groups II and III were further sub-divided into two groups of two and three rats each respectively (i.e. group IIA, 2 rats, group IIB 3 rats and group IIIA 2 rats and group IIIB 3 rats), which were orally administered with 200mg/kg and 400mg/kg of fixer effluents daily for 14 days and 28 days respectively. Thus group IIA rats were administered with low dose (200mg/kg) of fixer effluent for short term period of 14 days; group IIB rats were administered with high dose (400mg/kg) for long term period of 28 days. The average fixer effluent intake was 0.2ml/day for the low dose (200mg/kg) group and 0.42ml/day for the high dose (400mg/kg).

This experiment was proposed to be time and dose-dependent with same low dose (200mg/kg or 400mg/kg) being administered daily to Group II or III for 14 days and 28 days respectively. On the 15<sup>th</sup> day, two rats from group II and III each were sacrificed (using cervical decapitation



**Figure 1:** Photomicrograph of the liver of Wistar rats control group(x200) with normal hepatocytes(Arrow)

method), while the remaining three rats from group II and III each were sacrificed after 28 days and their livers harvested.

### Tissue Preparation

The moment the animals were sacrificed, they were immediately dissected and their livers stripped off and quickly fixed using Bouin's fluid and transferred into specimen bottles and kept frozen for 48 hours before undergoing normal processing such as dehydration, clearing and infiltration with melted paraffin. The liver tissues were fixed in paraffin wax, sectioned and placed on a hot water bath, thereafter, they were dried and dyed using hematoxylin and eosin (Ugwu et al., 2017; Drury and Wallingto, 1980). The photomicrographs were viewed using Nikon research microscope (Novex, Holland). The micrograph pictures were taken with high-resolution digital camera (DCM 510.5M Pixels, CMOS chip).

### Ethical Consideration

All methods adopted in this study was traditionally in line with the criteria and guiding principles for research involving animals as summarized in the "Guide for Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and circulated by the National

Institutes of Health (NIH) publication 86-23 revised (1985). The experiments were conducted after an ethical approval obtained from the Ethical Board of Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University.

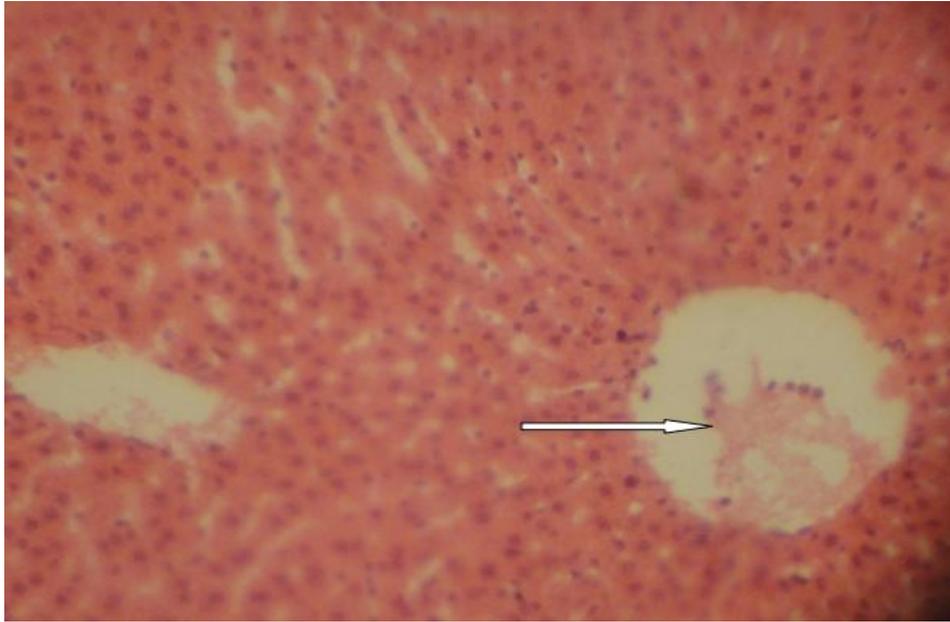
### RESULTS

Fourteen (14) Wistar rats were examined for the effects of fixer effluent on their liver. They were divided into control (group 1, n=4) and experimental (group II and III, n=5 each) groups.

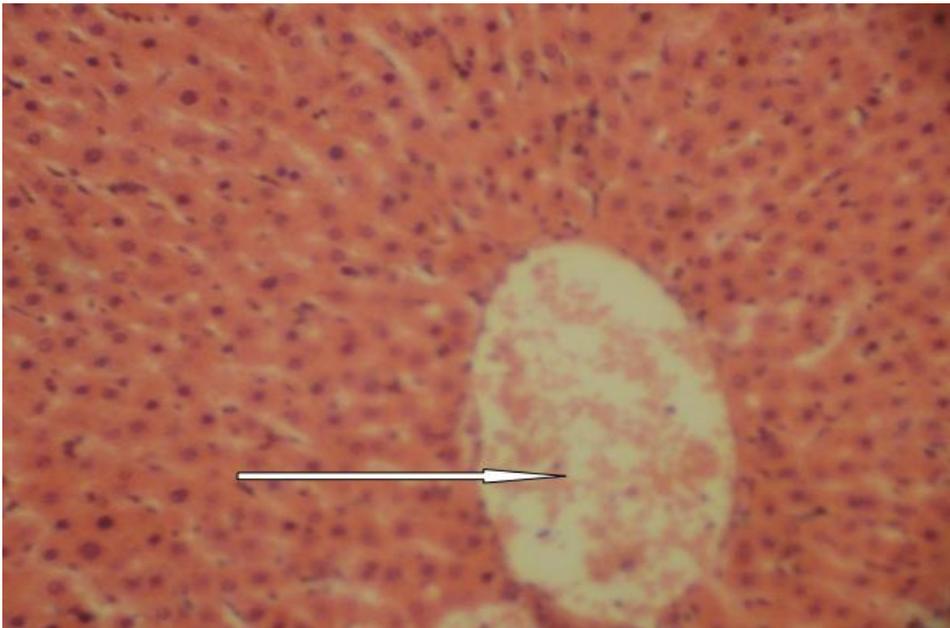
The result of the control group revealed normal histology of the Wistar rat's liver with hepatocytes clearly arranged in a lamina plate (Figure 1).

The Wistar rat's liver from group II and III that received two weeks (short-term) oral administration of low dose (200mg/kg) fixer effluent showed liver tissue with hypertrophied (distended) central vein with indication of edema within the vein and hepatocytes clearly arranged in the lamina plate (Figure 2).

The liver of the rats from the experimental groups that received 4 weeks (long-term) oral administration of the low dose (200mg/kg) fixer effluent, revealed normal hepatocytes, interstitial tissue and distended central vein with mild infiltrate of edematous fluid (Figure 3).



**Figure 2:** Photomicrograph of the liver of Wistar rats (x200) that received short-term oral administration of low dose (200mg/kg) fixer effluent with hypertrophied (distended) central vein with indication of edema within the vein and hepatocytes clearly arranged in the lamine plate(Arrow)

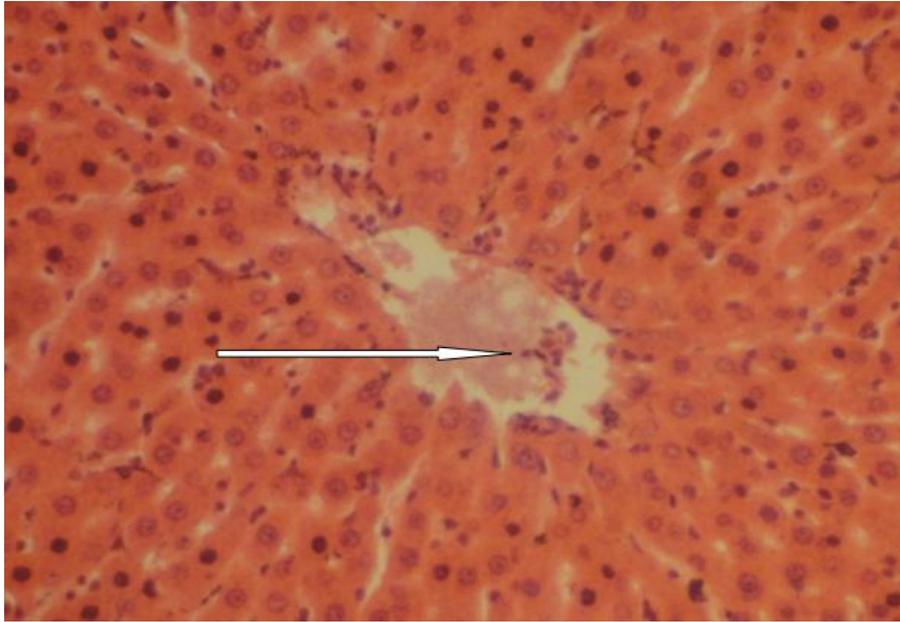


**Figure 3:** Photomicrograph of the liver of Wistar rats (x400) that received long-term oral administration of low dose (200mg/kg) fixer effluent with revealed normal hepatocytes, interstitial tissue and distended central vein with mild infiltrate of edematous fluid (Arrow).

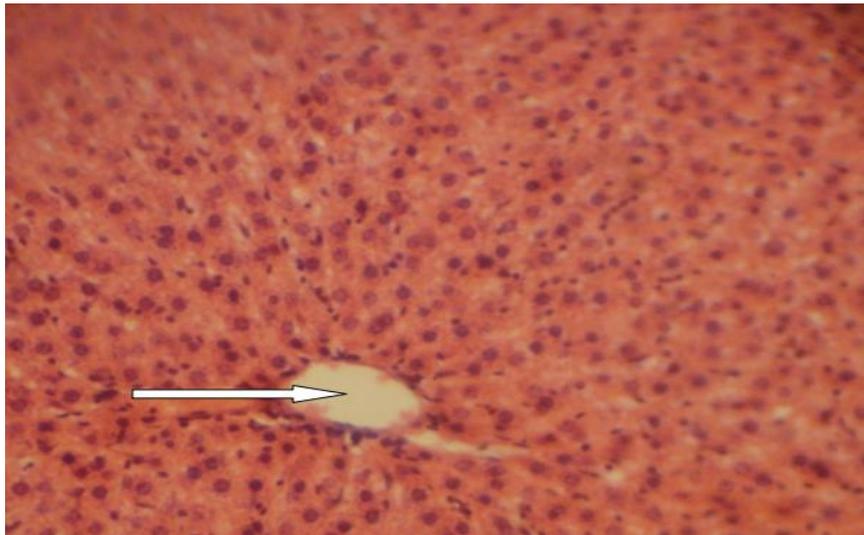
The Wistar rat's liver of the experimental groups that received 2 weeks (short-term) oral administration of high dose (400mg/kg) fixer effluent showed liver tissue with enlarged central vein with indication of edematous

infiltrate accumulation within the vein and normal hepatocytes clearly set on the lamine plate (Figure 4).

The experimental groups that received long term (4 weeks) oral administration of high dose (400mg/kg) fixer



**Figure 4:** Photomicrograph of the liver of Wistar rats that received short-term oral administration of high dose (400mg/kg) fixer effluent with enlarged central vein with indication of edematous infiltrate accumulation within the vein and normal hepatocytes clearly set on the laminae plate (Arrow).



**Figure 5:** Photomicrograph of the liver of Wistar rats (x400) that received long-term oral administration of high dose (400mg/kg) fixer effluent with normal histology, hepatocytes, interstitial tissue and central vein (Arrow).

effluent, showed normal histology, hepatocytes, interstitial tissue and central vein on photomicrograph (Figure 5).

## DISCUSSION

Many health centers discard their effluents into public

sewer systems with levels of inorganic compounds such as silver above allowed limits (Grigolletto, 2008). Additionally, such effluents discarded pose human and environmental threat. Our findings revealed that liver being a major metabolic organ in the body is vulnerable to toxicological effects of fixer effluent as it tries to convert it into a harmless chemical, thereby develop histopathological changes in the

liver. The study revealed that short-term administration of low dose fixer (200mg/kg) shows hypertrophied (enlarged) central vein with sign of edema within the vein and normal hepatocytes directly arranged in the lamina plate. Long-term administration of low dose fixer (200mg/kg) showed normal hepatocytes, interstitial and enlarged central vein with mild infiltrate of edematous fluid. The short-term administration of high dose (400mg/kg) fixer had histopathologic effect on the liver; a section of liver tissue shows hypertrophied (enlarged) central vein, sign of edematous infiltrate accumulation within the vein and normal hepatocytes distinctly arranged in the lamina plate. The long-term administration of high dose fixer (400mg/kg) shows normal histology, hepatocytes, interstitial tissue, central vein are normal. This revealed that fixer effluent had no effect on the hepatic cells (hepatocytes), but on the hepatic veins. Short term exposure to fixer effluent may cause histopathological changes of the liver of Wistar rat. The mechanisms behind the hepatotoxicity effects of fixer effluent in this study were beyond the scope of this study. This study reveals that fixer effluent caused hypertrophy of the central vein. The physiology, anatomy, nutritional, pathological and metabolic processes of Albino rats of Wistar strain was similar to that of humans, hence, the recruitment of these rats for this study.

## CONCLUSION

The present study showed that long and short-term oral administration of low dose fixer effluent, as well as short-term administration of high dose of fixer caused changes in the histological patterns of a healthy Wistar rats' liver. A few of the histopathologic effects were spotted in short-term low dose, long-term low dose and short-term high dose exposures of the rats.

## RECOMMENDATIONS

Based on the identified hepatotoxicity effects of radiographic fixer effluent in this study and the need to curtail perils to both the environmental and public health, we advocate that, adequate treatment of fixer effluent should be carried out by licensed agencies before disposal. Awareness campaign on the danger and the need for proper disposal of the fixer effluent should be intensified at all levels of government.

**Conflict of interest:** The authors declare that there is no conflict of interests regarding the publication of this manuscript.

## REFERENCES

- Adal A, Wiener SW (2018). Heavy Metal Toxicity. Medscape article ,updated 2018. Available from: <http://emedicine.medscape.com/article/814960-overview>. Retrieved on the 28th May, 2019.
- Bowen H (2007). Effect of Acetoacetic Acid on metabolic functions of accessory organs of the Gut. *J. Applied Toxicology*, 132: 183-87.
- Burkel WE, Low FN (1996). The fine structure of rat liver sinusoids, space of Disse and associated tissue space. *Am J. Human Ana.*, 118:769- 772.
- Cullinan P, Cannon J, Madan I, Newman T (1992). Occupational asthma in radiographers., 340: 1477.
- Drake PL, Hazelwood KJ (2005). Exposure-Related Health Effects of Silver and Silver compounds. A Review. *The Annals of Occupational Hygiene*. 49(7): 575-585.
- Drury RAB, Wallington EA (1980). *Carleton's Histological Techniques*. 5<sup>th</sup> edn., Toronto, Oxford, New York.
- Grigolletto A, Piala (2008). Environmental Contaminants and Treatment. *Environ. Health South Africa*, 15:75-97.
- Gunn C (1994). *Radiographic Imaging: A practical approach*. Churchillivingstone.
- Guyton C, Hall JE (2005). *Textbook of Medical physiology*. 11<sup>th</sup> Edition (p.54-66). Elsevier Philadelphia, Pennsylvania.
- Harper C (2003). Radiographic effluent management in Gaza Radiodiagnostic Centers, 25. 363 *Asian J* 183: 294-301.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54: 275-287.
- Mosby (2009). *Fixer*. Mosby's Medical Dictionary, 8<sup>th</sup> edition, Elsevier.
- National Institute for Health (NH)(1985). *Guide for the care and use of Laboratory Animals*. NH Publication 86-23 revised 1985.
- Ogolodom MP (2015). *Essentials of Medical Image Processing and Photography for Radiographers, Radiographic Technicians and Darkroom Assistant*, Godwin publisher, Port Harcourt, Nigeria (Chapter Six).
- Thomas F, Leesson S (1965). *Histology Textbook*. British Publishers Saunderland., p 234.
- Tseke K, Chow W (2000). Exposures and their control in radiographic film processing in British Columbia. *Photo Science*. 2: 16-17.
- Ugwu AC, Dimkpa U, Ijabor BO, Ewunonu E O, Eteudo A N(2015). Nephrotoxicity of radiographic fixer effluent in Wistar rats. *J. Mol. Pathophysiol.*, 144-148.
- Warren B (2001). *Photography*. 2<sup>nd</sup> ed. Delmer Thomson Learning. Albany, New York.
- Wynmer H (2000). Sodium thiosulphate, side effects and allergic symptoms on albino rat. *J Toxicolog. Environ. Health*. 3: 33-34.