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Cardiopulmonary Reactions In Wistar Rats Exposed To X-Ray Film Developer Solutions.

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ABSTRACT

Diseases affecting the respiratory and cardiovascular systems are on the increase in many developing countries and often lead to death. Life style and work environment tend to contribute to the ugly development which is worsened by lack of the right drugs for treatment. Empirical observations showed that the disease was seen more in people exposed to chemical fumes. Unfortunately, majority of the radiology practices in developing countries including Nigeria is darkroom-based, thus exposing radiographers and darkroom staff to x-ray film processing chemical fumes. This experimental research was aimed at investigating the cardiopulmonary reactions in wistar rats exposed to x-ray film developer solutions of varying concentrations. Eighteen apparently healthy wistar rats of 20-24week weighing between 208g and 210g were put in three groups A-C of 6rats each. Group A was the control group while groups Band C were the experimental groups. The rats in the experimental groups were exposed to varying concentrations of the x-ray film developer solution fumes for periods ranging from 15-30days (1st -30th June, 2014) while the rats in the control group were not. At the end of each desired period, two rats from each group were randomly selected, painlessly sacrificed and the lungs and hearts harvested and sent to the medical laboratory sciences department of the Nnamdi Azikiwe University Teaching Hospital for examination and analysis. Micrographs of the lungs tissues in the experimental groups showed histological changes of tissue injury evidenced by macrophage infiltration, distortion of interstitial tissue architecture, thickening of basement membrane, tissue fibrosis and formation of pleomorphic nuclei which are inflammatory responses indicating cellular injuries. The observed histological changes increased with the concentration of the developer solution and the duration of exposure to the developer solution. Micrographs of the hearts in the experimental and control groups showed normal histological appearances. When the changes in the lungs are extrapolated to a 70kg human subject, these histological changes would manifest within twenty-five year of exposure to the developer solution.

Keywords: X-ray film developer solution, Wistar rats, Lungs, heart, cardiopulmonary reactions.

INTRODUCTION

The heart and the lungs are two vital organs in the body whose state of health is very critical to life. Injuries to these organs often end fatally without prompt and adequate care being taken. Unfortunately, many chemical fumes including fumes from x-ray film processing solutions were reported to be injurious to the lungs (Gordon, 1984; 1987; 1989; Herwitt, 1993; Matthew and Yehunda, 1986). Some cardiac problems were also reported to be associated with exposure to chemical fumes (William, 1978; Connaughton, 1993; Smedley and Coggon, 1996; Smedley et al., 1996). In mammals, the respiratory system consists of the nasal cavity, the nasal and oral portion of the pharynx, the larynx, trachea, bronchi and the bronchioles and alveoli in the lungs where gas exchange occurs (Roger and Peter, 1973). Inhaled gases/fumes move from the upper airways and reach the alveoli in the lungs. As the gas/fume passes deeper into the respiratory tract, more soluble gases are adsorbed and particles are deposited on the airways and alveoli. With deposition of particles diffuse changes occur that affect the lining of the airways-the epithelium and the structure of the bronchioles. This can result in symptoms of chronic obstructive pulmonary disease (COPD) such as emphysema, chronic

bronchitis, asthma, idiopathic pulmonary fibrosis (USDHHS, 2004). In the transportation and exchange of gasses, there is a functional relationship between the heart and the lungs. The endocardium of the heart consists of squamous epithelial cells with oval nuclei and forms the inner lining of the chambers of the heart and is related to blood vessels. The pulmonary trunk carries deoxygenated blood from the right ventricle to the lungs via the right and left pulmonary arteries while the pulmonary veins transport oxygenated blood from the lungs to the right atrium of the heart for distribution to the body. The integrity of this functional relationship between the heart and the lungs is very essential for maintenance of life.

In the production of diagnostic radiographic images chemical processing is always involved in radiology facilities where darkroom radiography is in practice as is the case in many developing countries like Nigeria. The production of radiographs involves two major processes, namely the x-ray exposure of the film emulsion to form invisible/latent images and the chemical processing of the film to obtain the visible/permanent images. The chemical processing of radiographs also involves two main processes-the development and the fixing of the radiograph. The development process brings out visible images from the latent images formed during the x-ray exposure of the film emulsion while the fixing process fixes the images permanently on the film emulsion base.

The main components of the developer solution used for the development of radiographic films are the Metol hydroquinone or Phenol hydroquinone and other agents such as gluteraldehyde, sodium hydroxide and potassium and sulphur compounds. Gluteraldehyde has predilection for the respiratory system (Cui et al. 1996) and sodium hydroxide was reported to have poisoning effect on the heart (NIH, 2014). Burge (1989) observed a range of severe effects in individuals exposed to these processing chemicals even at very low concentrations. Haemorrhage, alveolar wall thickening, pulmonary vessel dilatation and inflammatory cell invasion of the lungs were also observed (Neelam et al., 2011). Other symptoms observed in individuals exposed to film processing chemicals include severe headache, gastrointestinal tract and urinary problems, peripheral artery spasm, chest pains, nasal congestion, catarrh, nausea, joint pains and unexpected fatigue (William, 1978; Matthew and Yehunda, 1986; Spicer et al., 1996; Nallon, 2000).

The observation of these symptoms has serious implications for developing countries where darkroom radiography is practiced by more than ninety percent (>90%) of the radiology facilities. This research was therefore, aimed at investigating the effects of x-ray film developer solutions of varying concentrations on the lungs and hearts of wistar rats. The result of the study may help to draw the attention of health authorities and other stake holders to the need for speeding up transition to filmless/digital radiography as is now the case in the developed countries. Empirical studies revealed higher incidence of respiratory problems among radiographers and darkroom personnel (Liss et al., 2003).

MATERIALS AND METHODS

Ethical approval for this experimental research was obtained from the Research and Ethical Approval Committee of the Faculty of Health Sciences and Technology of Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria. The research was carried out between 1st and 30th June, 2014.

Eighteen (18) apparently healthy wistar rats of 20-24 weeks and weighing between 208g and 210g were used. These rats were obtained from the animal farm of Nnamdi Azikiwe University, Nnewi Campus. These rats were randomly divided into three groups (A-C) of six rats each-groups B and C as the experimental groups and group A as the control group. Each group of rats was put in a labeled metal cage and observed for one week to acclimatize in their new environment before the start of the study.

Experimental Procedure

A full concentration of the developer solution (0.05g per cm^3 was prepared by dissolving the 500g packet of developer powder in 10litres (10,000cm^3) of water. 5000cm^3 of the prepared developer solution was then diluted by equal volume of water to

obtain a developer solution with half the concentration of the original solution(0.025g per cm^3).

Wistar rats in the experimental group B were exposed to the fumes from the full concentration developer solution contained in a plastic bowel placed close to the cage harbouring them. By the same method, the rats in the experimental group C were exposed to the fumes from the developer solution of half concentration. The rats in the control group, A were not exposed to any developer solution fumes. The cages harbouring the rats were kept in different rooms of a house. The rats were fed with Vital Feeds Growers palletized for feeding the rats and water as prescribed by the manager of the animal house from where they were obtained. Each of the rooms housing the rats was lit by a 15watt energy bulb as advised by the manager of the animal house.

On the 15^{th} day of the experiment, two rats were randomly selected from each group for dissection. Before dissecting the rats, each was weighed and their weights noted. Each of the rats was then anesthetized by placing it in a bell jar with a wire mesh floor over gauze moistened with chloroform and observed for signs of decreased motility and unsteady gait for about 20seconds. Each rat was then brought out of the bell jar and painlessly sacrificed. With gloved hands, the proper incision was made on the midline of the ventral aspect from the jugular notch through thoracic region to the abdomen. The lungs and heart of each rat was then harvested, preserved in 10% formalin in a plain sample bottle and sent to the medical laboratory sciences department of Nnamdi Azikiwe University, Nnewi Campus for analysis.

On that same 15^{th} day, two rats were randomly selected from each of the experimental groups (B and C) for pulmonary aspiration with the respective developer solutions. Before aspirating the rats with the developer solutions, the rats were labeled B1, B2, C1, and C2 and weighed. To aspirate the rats with the developer solutions, each rat was anesthetized using the same procedure described earlier for the rats sacrificed above. Each rat was then brought out of the bell jar. Using a 5ml syringe each rat was aspirated with 0.5ml of the solution: rats B1 and B2, with the full strength developer solution and rats C1 and C2, with the half strength developer solution. To instill the solution, the mouth of the rat was opened. The tongue was pulled to a side using a swap stick and the solution was introduced to pass down through the pharynx. The aspirated rats were then kept on a slab and observed to recover from the effects of the anesthesia and were put back into their respective cages where they stayed and continued with the non-aspirated rats in the group.

On the 30^{th} day (end of the experiment) all the rats in each group were painlessly sacrificed after anesthetizing with chloroform as described earlier for the rats sacrificed on the 15^{th} day.

Tissue collection

With a surgical blade a ventral midline incision was made from the jugular notch through the thoracic region to the abdomen of each rat. The lungs and heart were harvested and each preserved in a vial of 10% formalin. The organs were labeled according to their groups and mode of exposure to the developer solution (ie inhalation only or inhalation plus aspiration). The specimens were then sent to the medical laboratory sciences department for examination and analysis of the histological and morphological changes that might have occurred in the lungs and hearts of the rats.

Tissue Preparation and Processing

The lung and heart tissues were separately processed using standard operative procedures and embedded in molten paraffin wax. The embedded tissues were then mounted on wooden blocks, sectioned with microtome knife and stained with Erhlich's haematoxylin and Eosin staining method (Avwioro, 2011). The stained slides were then cleared in xylene and mounted in dibutylphthalate polystene xylene (DPX).

Microscopy and photomicrography

Microscopic examination of the cut sections was carried out using Swift binocular microscope with in-built lighting system. Sections with striking features were selected

photomicroscope with coloured films. Results were expressed in terms of observed physical/behavioural changes in the rats and histological changes in heart tissues, the alveolar cells, epithelial lining of the bronchioles, bronchi and other cellular components of the lungs and heart.

RESULTS

The results of the study was expressed in terms of observed physical/attitudinal changes in the rats and histological changes in the cardiac and lung tissues and other cellular components of the heart and lungs.

Slight loss in weight, decreased social activities and poor response to feeds and water were observed in the experimental rats towards the end of the experiment. Photomicrographs of the lungs of the rat from the experimental group B (exposed to full concentration of developer solution) sacrificed on the 15th day showed infiltrates of macrophages and thickening of basement membrane (Plate 1) while that of the sacrificed rat from experimental group C (exposed to half strength developer solution) showed distorted interstitial tissue architecture, mild infiltrate of macrophages with pleomorphic nuclei (Plate 2). The photomicrographs of the heart tissues in the experimental and control groups of rats sacrificed the same 15th day of the experiment showed normal histological appearances for both the full and half concentrations of developer solutions(Plates 3, 4, and 5).

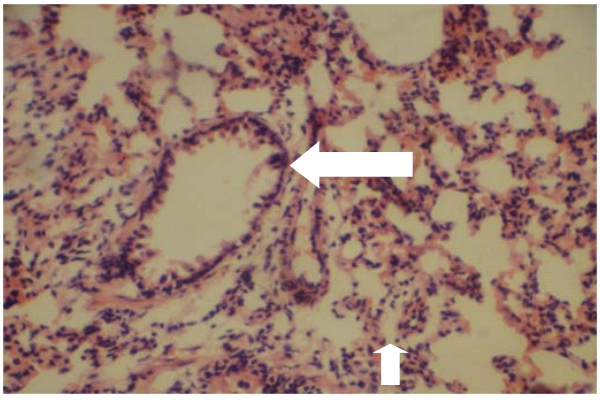


Plate 1: Photomicrograph of the lungs of wistar rat sacrificed on the 15th day of inhalation of full concentration of developer solution fumes (Mag. X100) showing infiltrate of macrophages (Small arrow) and thickening of basement membrane (Big arrow).

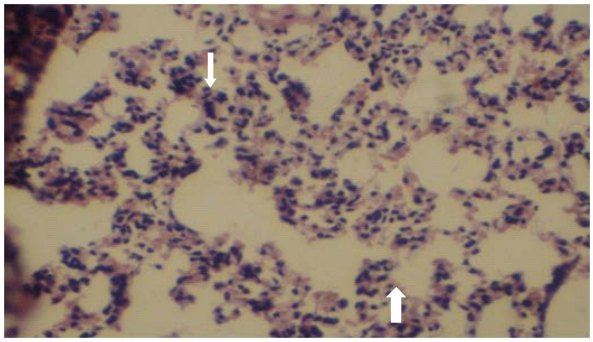
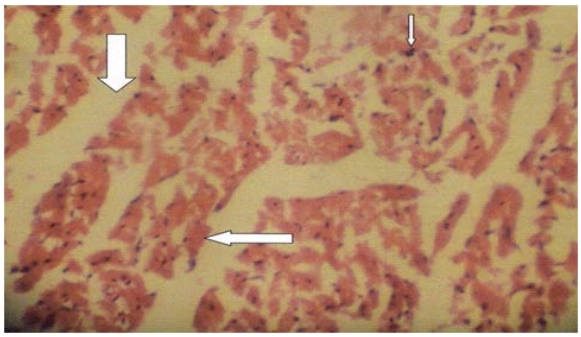
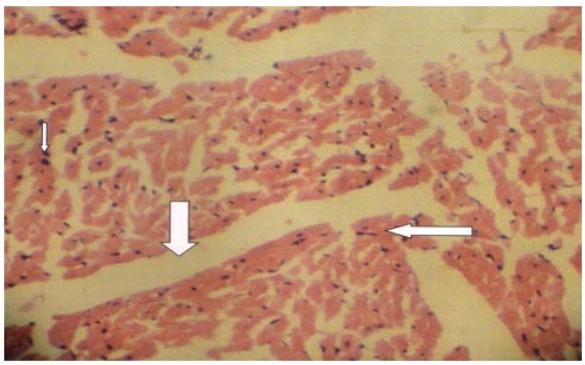


Plate 2: Photomicrograph of the lungs of wistar rat sacrificed on the 15th day of inhalation of half strength concentration of developer solution fumes (Mag. X100) showing mild infiltrate of macrophages with pleomorphic nuclei (thin arrow) and distorted interstitial tissue architecture (thick arrow).





Photomicrograph of the heart tissues of wistar rats sacrificed on the 15th day of exposure to: full strength concentration of developer solution fumes (Plate 3), half strength developer solution fumes (Plate 4) and no developer solution fumes –the control group (Plate 5) (Mag. X100), all showing normal histological appearances.

Key: Nucleus (small arrow), Cardiac myocardium (narrow arrow), Blood vessel (thick arrow).

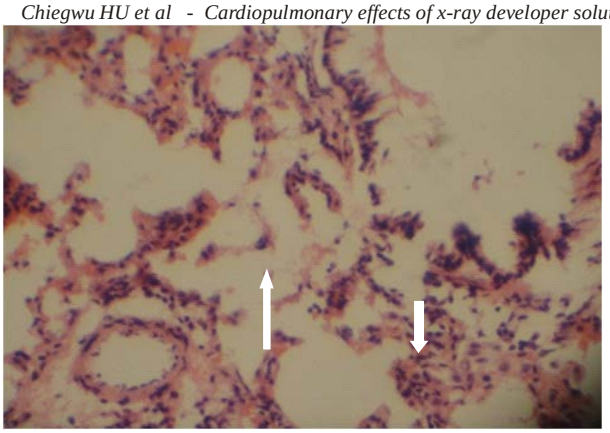


Plate 6: Photomicrograph of the lungs of wistar rat sacrificed on the 30th day after exposure to full strength concentration of developer solution fumes by both inhalation and aspiration (Mag. X100) showing distorted interstitial tissue architecture (Big arrow) and mild infiltrate of macrophages (small arrow).

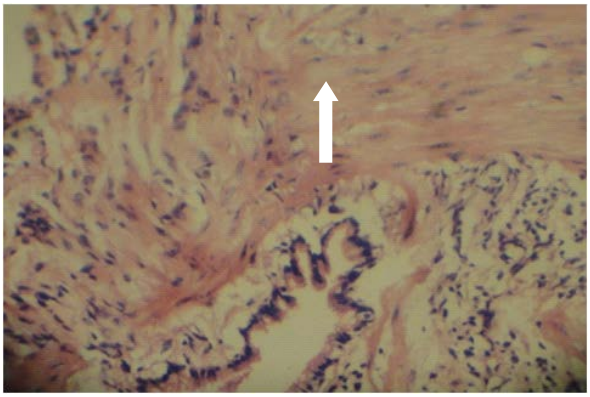


Plate 7: Photomicrograph of the lungs of wistar rat sacrificed on the 30th day after exposure to full strength concentration of developer solution fumes by inhalation only (Mag. X100) showing distorted interstitial tissue architecture and infiltration of fibrous tissue within the stroma (Arrow).

The lung tissues of the rats from experimental group C that were exposed to half concentration of the developer solution by both inhalation and aspiration (rats C1 and C2) and sacrificed on the 30th day showed distorted interstitial tissue architecture and mild infiltrate of macrophages (Plate 8) but the lung tissues of the rats exposed to the same developer concentration by inhalation only revealed infiltrate of macrophages and signs of inflammatory oedema (Plate 9).

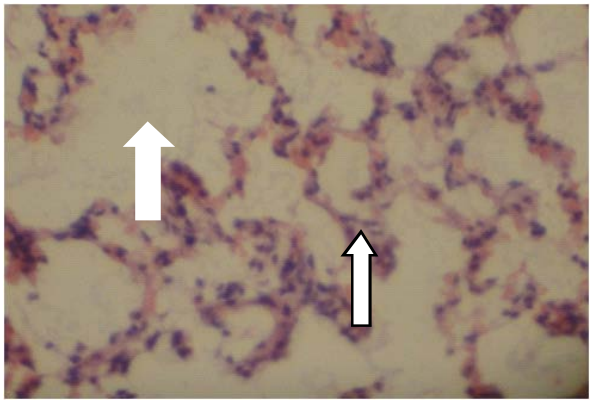


Plate 8: Photomicrograph of the lung tissues of wistar rat sacrificed on the 30th day after exposure to half strength concentration of developer solution fumes by both inhalation and aspiration (Mag. X100) showing distorted interstitial tissue architecture (Big arrow) and mild infiltrate of macrophages (Small arrow).

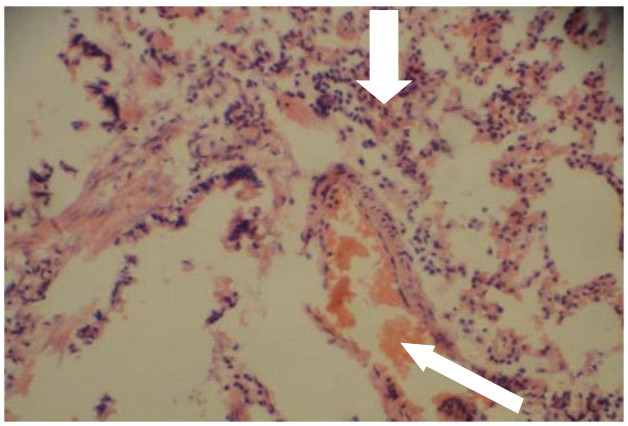


Plate 9: Photomicrograph of the lung tissues of wistar rat sacrificed on the 30th day after exposure to half strength concentration of developer solution fumes by inhalation only (Mag. X100) showing infiltrate of macrophages (thick arrow) and signs of inflammatory oedema(thin arrow).

As was the case for the rats sacrificed on the 15th day of the experiment, photomicrographs of the cardiac tissues of rats from both the experimental and control groups sacrificed on the 30th day showed normal histological appearances.

DISCUSSION

Insults to either the lungs of hearts are most of the times serious and require urgent and adequate attention. Such injuries are often of a chronic nature and life threatening. When the two

organs become simultaneously involved in injury/injuries, the economic implications are usually much and the prognosis is often poor.

The poor response to feeding, loss of weight and sluggish activities observed in the experimental groups of rats are possibly signs indicative of the presence of a problem with the rats. Possible confirmation of this was the observed distortion of interstitial tissue architecture, infiltrate of macrophages and thickening of basement membrane which are defense responses initiated by the lungs and respiratory tract tissues to respond to inflammatory processes/injury caused by the inhaled developer fumes. The macrophages are large, mobile, highly phagocytic cells that become mobile when stimulated by inflammation and migrate to the affected area. The process of acute inflammation is a protective measure initiated by certain tissue cells, mainly resident tissue macrophages, mastocytes, histiocytes etc aimed at removing injurious stimuli and initiate a healing process. Whenever there is infection or cell injury these defense tissue cells are activated and release inflammatory mediators that are responsible for the clinical signs and symptoms of inflammation. The findings in this study are similar to the findings by Kheradmand et al (1994) and Geiser (2000) in lungs exposed to formaldehyde inhalation.

The degree of the observed pulmonary histological changes noted in this study was related to the concentration of the developer solution, the period of exposure and the quantity of the developer solution entering the lungs. The distortion of interstitial tissue architecture and formation pleomorphic nuclei agreed with the mechanism of excavation and desquamation of surface epithelium and derangement leading to ulceration of alveoli reported by Njoya et al (2009). The absence of any of the histological changes in the control group implicated the developer solution as the cause of those histological changes. The findings this research may provide explanations for the symptoms found among radiographer in New Zealand exposed to fumes from darkroom film processing chemicals reported by Spicer et al(1986) and also the respiratory abnormalities among photographic developers reported by the quest for filmless and digital radiography which is now practiced in many advanced countries of the world. But for many developing counties including Nigeria, darkroom radiography is practiced in more than 80% of radiology centres. Empirical observations also reveal more respiratory problems among radiology workers than their counterparts in other professions.

A very striking and surprising finding was the normal histological features of the cardiac tissues of the rats found in both the experimental and control groups. With the functional relationship between the lungs and the heart, the observation of histological changes in the lung tissues may make one suspect the same in the cardiac tissues. The normal cardiac histological finding in this study contradicted the low blood pressure, cardiac atrophy and collapse due to exposure to processing chemicals reported by Kristek et al (2013) and NIH (2014). It equally contrasted the findings of cardiac arrhythmia and tachycardia reported by Spicer et al., (1986), Gordon (1987; 1989), Connaughton (1993) and Smedley and Coggon (1996). The explanation for the different finding in this study may be the short period of exposure to the developer solution. The other researchers carried out their research for long periods simulating what is obtained in the clinical settings.

One inference to make about the finding in the lungs and the heart of the exposed rats is that the lung tissues are more sensitive to the processing chemical fumes than the cardiac tissues. Another explanation may be that the defense cells of the lungs check-mated the arriving insult, preventing it from reaching the heart within the short period of the study. The insult would only reach the heart if the insulted persisted for a long time when the defense mechanism of the lungs might have become weak.

When the duration of the experiment(period of exposure of the rats to the developer solution) and the concentration of the developer that caused the histological changes to the lungs were extrapolated to a 70kg human subject, it was found out that such effects would appear in about 25years. By that time the cardiac issues might have been affected. The implication is that in countries like Nigeria

where the official working period is 35years, a darkroom worker will complete the remaining 10year with respiratory and possibly cardiac problems and will retire from service miserably.

CONCLUSION

Various histological changes depicted by infiltrates of macrophages, distortion of interstitial tissue architecture, pulmonary oedema, alveolar tissue fibrosis and formation of pleomorphic nuclei were observed in wistar rats exposed to varying concentrations of x-ray film developer solution. The severity of the histological changes was related to the concentration of the developer solution and the period of exposure to the solutions. Extrapolating the results of the study to humans, such changes could manifest within 25years of exposure at work place.

In view of the likely consequences of exposure of radiology workers (radiographers and darkroom technicians) to the x-ray film developer solutions efforts should be made to hasten the move from darkroom radiography to filmless or digital radiography in developing countries. Also the hours and days of duty for radiographers and darkroom technicians should be reduced as to reduce the period of exposure to film processing chemicals and hence the probability of respiratory and/or cardiac problems. Also the use of automatic film processors should be encouraged because exposure to developer solutions via automatic processors is less than for manual processors.

Limitations Of The Study

It was not possible to study the force of contraction of the heart which would have enabled us know the heart rate was the same for the rats in the experimental and control groups. This would have shown whether there were cardiac palpitations, arrhythmias and/or tachycardia which were reported by some authors. Also there was no blood analysis to check for changes in the composition and chemistry of the blood following exposure to the developer solutions. Our research was only on the histological changes.

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