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Cardiotoxic Assessment of Radiographic Developer Effluent in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ACU and DF designed the study, wrote the protocol and wrote the first draft of the manuscript. Author UD was the correspondent author, managed the literature searches, carried out image analyses and processing and editing of the manuscript. Authors ANE, SOM, NNNO, LCA and RCU managed the literature searches and the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To demonstrate the histopathological changes in cardiac tissues of Wistar rats following exposure to developer effluent.
Study Design: A case control study.
Place and Duration of Study: Department of Radiography, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

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Methodology: Eighteen young Wistar rats of weighing 140-220 g were used for the study. The animals were divided randomly into three groups of 6 rats each based on the dose of developer effluent administered to them – i.e. control group I (0 dose) and experimental groups II (lower dose, 200 mg/kg) and III (higher dose, 400 mg/kg) respectively. The groups were further classified as either A or B sub-groups of three rats each, depending on the duration (14 or 28 days) of effluent administration. The effluent administration was done by oral gavages. Image processing and analyses were done using ImageJ software (version 1.49) to obtain particle count, circularity of particles, number of nuclei and number of connective tissue spaces.
Results: Normal heart histology was observed in the control group. In contrast, lower dose of developer effluent administered for two weeks caused mild edema on cardiac tissues and occasional loss of myocardial fibers. Fourteen days of effluent administration at higher dose indicated alteration in the cardio-myocytes, necrosis of intercalated discs, moderate distortion and enlargement of cardio-myocytes structure and edema. Administration of lower dose developer effluent for 28 days caused severe distortion and enlargement of the cardio-myocytes structure with destruction and loss of intercalated disk. The higher dose of effluent caused severe distortion and enlargement of the cardio-myocytes, loss of intercalated discs, and irregular nuclei after 28 days of administration.
Conclusions: The present study which indicated adverse effects of acute/chronic and long-term/short-term exposures to sub-lethal doses of developer effluent on Wistar rats' heart tissues suggests the need for proper management and disposal of developer effluent.

Keywords: Environment; workplace; exposure; radiography; histopathology.

1. INTRODUCTION

Radiographic developer is a very important solution widely used in the photographic and X-ray film processing to convert latent images to visible images. There is little information on the hazards of the chemical. However, it has been reported to cause harmful effects if inhaled or accidentally swallowed. Similarly, it has been shown to liberate toxic gases which cause irritation to the eyes, skin and the gastrointestinal tract as well as cause damage to the kidneys [1]. After its decomposition, developer produces substances such as carbon monoxide, carbon dioxide and oxides of sulphur, which are hazardous [1]. The exhausted waste of the radiographic developer is known as developer effluent and is generated during radiographic processing and contains organic and inorganic compounds, which are reported to be toxic to the environment (soil, water) and food in cases where they are inappropriately disposed of [2].

There is paucity of data on developer effluents produced in Nigeria. However, a preliminary survey on the developer consumption level in radiography and photography centers in Calabar, Cross River State, Nigeria, puts the developer effluent production at approximately 16,000 liters annually [1]. There are clear legal rules that give guidance for the proper discharge of these effluents in developed countries; however this situation is not common in developing countries [3]. Presently there is no legislation on the management of the radiographic effluents in Nigeria. This means that many photographic and radiographic/health centers including teaching and research institutions dispose these effluents into streams or public sewer systems without previous treatment or recycling. Consequently, the effluents are discarded into the environment with a high level of inorganic compounds, high chemical oxygen demand (COD) and hydrogenic potential (pH), total dissolved solids concentration, chlorides, sulfates and turbidity that are over allowed limits [2,4]. Exposure to harmful and toxic substances is known to likely occur through the diet, from medications, the environment and workplace [5]. Such exposures may pose great danger to the human body, particularly to organs associated with transport and blood circulation such as the heart.

To the best of our knowledge, no previous study has reported toxic effects of radiographic developer effluent on heart tissues. The present study therefore is aimed at investigating the histopathologic effects of developer effluent exposure to the heart tissues of Wistar rats.

2. MATERIALS AND METHODS

2.1 Animals

Eighteen apparently healthy Wistar rats weighing 140-220 g were used. They were housed in the

animal house of the Department of Human Anatomy, Nnamdi Azikiwe University, Nnewi Campus, under standard conditions (29±2℃ temperature, 40-55% humidity, good ventilation) and had free access to water and diet (normal rat chow). They were acclimatized for two weeks before the start of the experiment.

2.2 Test Chemical

The original product, a commercially prepared developer (a chemical used in processing photographic or x-ray films) was purchased from Begood Manufacturing Company Ltd, China. The main components of the developer are hydroquinone, sodium carbonate, sodium sulfite, potassium bromide and water [6]. The content of the exhausted developer effluent, the liquid waste material generated from radiographic processing, include hydroquinone and its oxidation products, carbonate ion, bromide ion, COD. It has a $_{P}$H of 10.4. The lethal dose (LD50) concentration of the developer effluent was calculated as 2450 mg/kg body weight using the formula: $LD_{50} = \sqrt{a \times b}$ (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 developer effluent was carried out at the Faculty of Pharmacy and Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus according to the method employed by Lorke [7]. The concentrations of the developer effluent used for the experiment were sub-lethal doses of 200 mg/kg (lower dose) and 400 mg/kg (higher dose) of body weight.

2.3 Experimental Design

The present experiment was designed to be time and dose-dependent. The animals were divided randomly into three groups of 6 rats each based on the dose of developer effluent administered to them – i.e. control group I (0 dose) and experimental groups II (lower dose, 200 mg/kg) and III (higher dose, 400 mg/kg) respectively. The groups were further classified as either A or B of 3 rats each depending on the duration (14 or 28 days) of effluent administration. Thus control groups IA and IB were administered with distilled water for 14 and 28 days respectively; group IIA rats were administered with lower dose (200 mg/kg) of effluent for a short term period of 14 days; the group IIB rats were administered with the lower dose of effluent for a long term period of 28 days; group IIIA rats were administered with higher dose (400 mg/kg) of effluent for short term period of 14 days; and group IIIB rats were administered with higher dose of effluent for long term period of 28 days. The effluent administration was done by oral gavages. The average developer effluent consumption was 0.2 ml/day for the lower dose group and 0.42 ml for the higher dose group. After 14 days, three rats from groups IA, IIA and IIIA were sacrificed (using the chloroform inhalation method), while the three rats from each of the remaining groups, IB, IIB, IIIB, were sacrificed after 28 days and their hearts harvested.

2.4 Tissue Preparation

As soon as the animals were sacrificed, they were quickly dissected and their hearts removed and immediately fixed in a fixative (10% formol – saline) for 24 hrs and transferred into specimen bottles, labeled and kept frozen for 48 hours before undergoing routine processing (dehydration, clearing impregnation and infiltration with melted paraffin). The heart tissues were embedded in paraffin wax, sectioned at 3 µm placed on a hot water bath, after which they were dried and stained by Cole's hematoxylin solution and 1% eosin solution. The photomicrographs were observed using research microscope (Leica DM 750). The micrograph pictures were taken with digital camera (DCM 510.5M Pixels, CMOS chip) connected to the microscope. All the tissue preparations and observations were done by the same research personnel.

2.5 Ethical Consideration

All procedures used in this study conformed to the criteria and guiding principles for research involving animals as outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985) [8]. The experiments were carried out following the ethical approval of the Ethical Board of Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University.

2.6 Image Analysis

All image processing and analyses were done using ImageJ software (version 1.49). The ImageJ software is a public domain, Java based image processing program designed for processing and analyzing scientific multidimentional images. The image analyses were done within the region of interest (ROI) to

determine color thresholds, cell counts, total area of ROIs, and percentage area of particles, mean gray value, integrated density of gray values and shape (circularity) of objects, is summarized in the table of results. Data were expressed as means, percentages and in microns.

3. RESULTS

The histological findings revealed that the techniques used for tissue preparation were successful in all sacrificed rats with less technical difficulties. None of the rats died before the last day of experiment. Every histological finding or change observed and stated in this study was identified either in all the rat tissues of each group or at least in two of the three rat tissues, however, only the clearest slides were chosen. All the rats in control groups IA, IB indicated same normal cardiac morphology; two of the three rats in groups IIA, IIB, IIIB indicated same histological findings respectively; and in group IIIA, all the three rat tissues indicated same findings.

The cardiac histology of the control rats revealed a normal appearance showing normal and centrally arranged nucleus, the intercalated discs and connective tissues also appeared normal and the cardiac muscle fibers are well arranged (Fig. 1). The image analysis showing the color

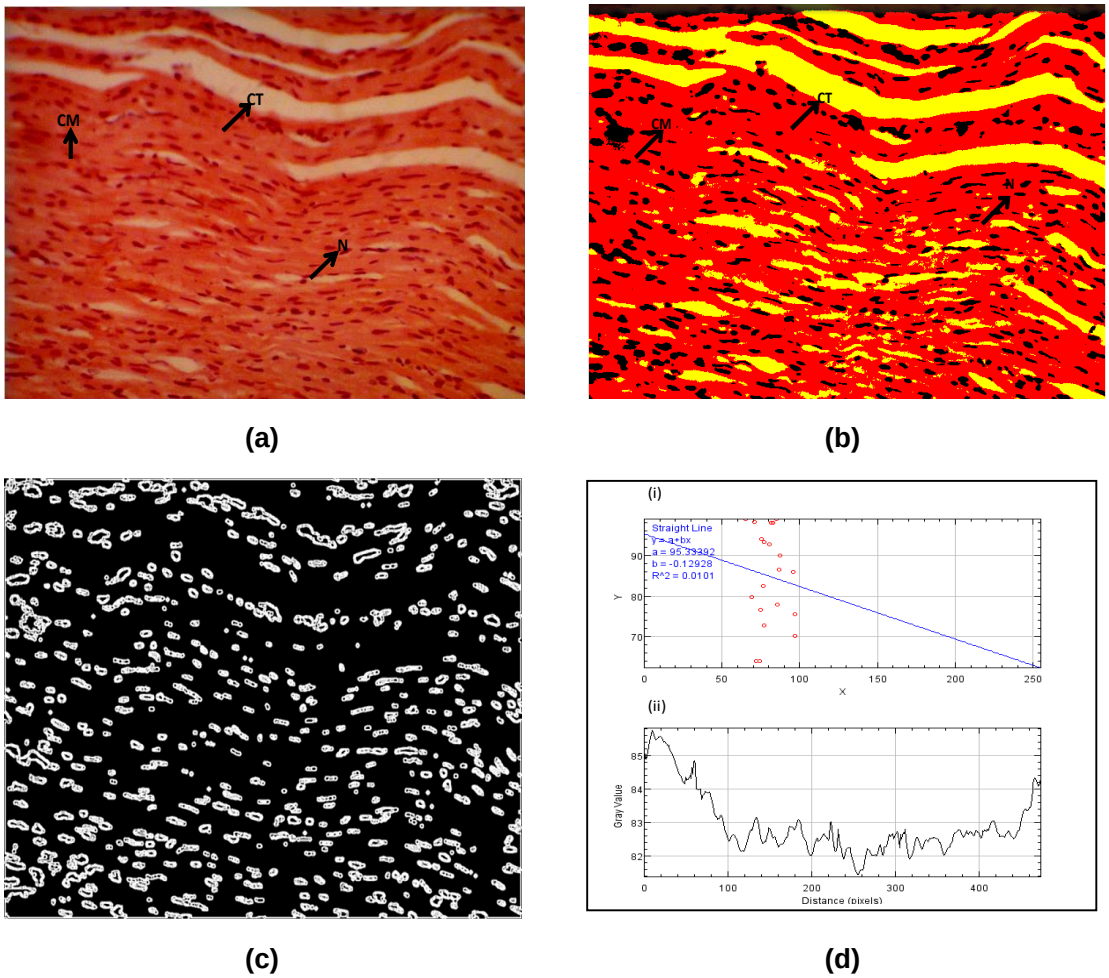

Fig. 1. (a) Photomicrograph showing normal architecture of the heart of group I (control) rat (H and E Stain x400); (b) Representation of the image color correction and improvement application; (c) Representative image of an analyzed field showing threshold color application; (d) Graphical representation of the cardiac histology (i) straight line plot showing the calibration of the image intensities within the region of interest (ii) A profile plot of particles within the region of interest. The nuclei (N), the connective tissues (CT) and cardiac muscle fibers (CM) appeared normal and well arranged

threshold, particle count, area of region of interest, % area of particles within the region of interest, mean gray value, integrated density of gray values, shape (circularity) of particles, number of nuclei, and number of connective tissue (CT) spaces, is summarized in the table of results (Table 1).

The cardiac histology revealed significant alterations in the histological profile of group IIA (administered with 200 mg/kg of effluent for 14 days) when compared to the control group. The histological findings include presence of mild edema and occasional loss of myocardial fibers (Fig. 2). Results of the image analysis (Table 1) indicated that group IIA had greater particle count (n = 839), and greater number of nuclei (n = 490) and CT spaces (n = 43) compared to the control (particle count, n = 452; nuclei, n = 185; CT spaces, n = 21) and the other groups. Other data of the image analysis are as summarized in Table 1.

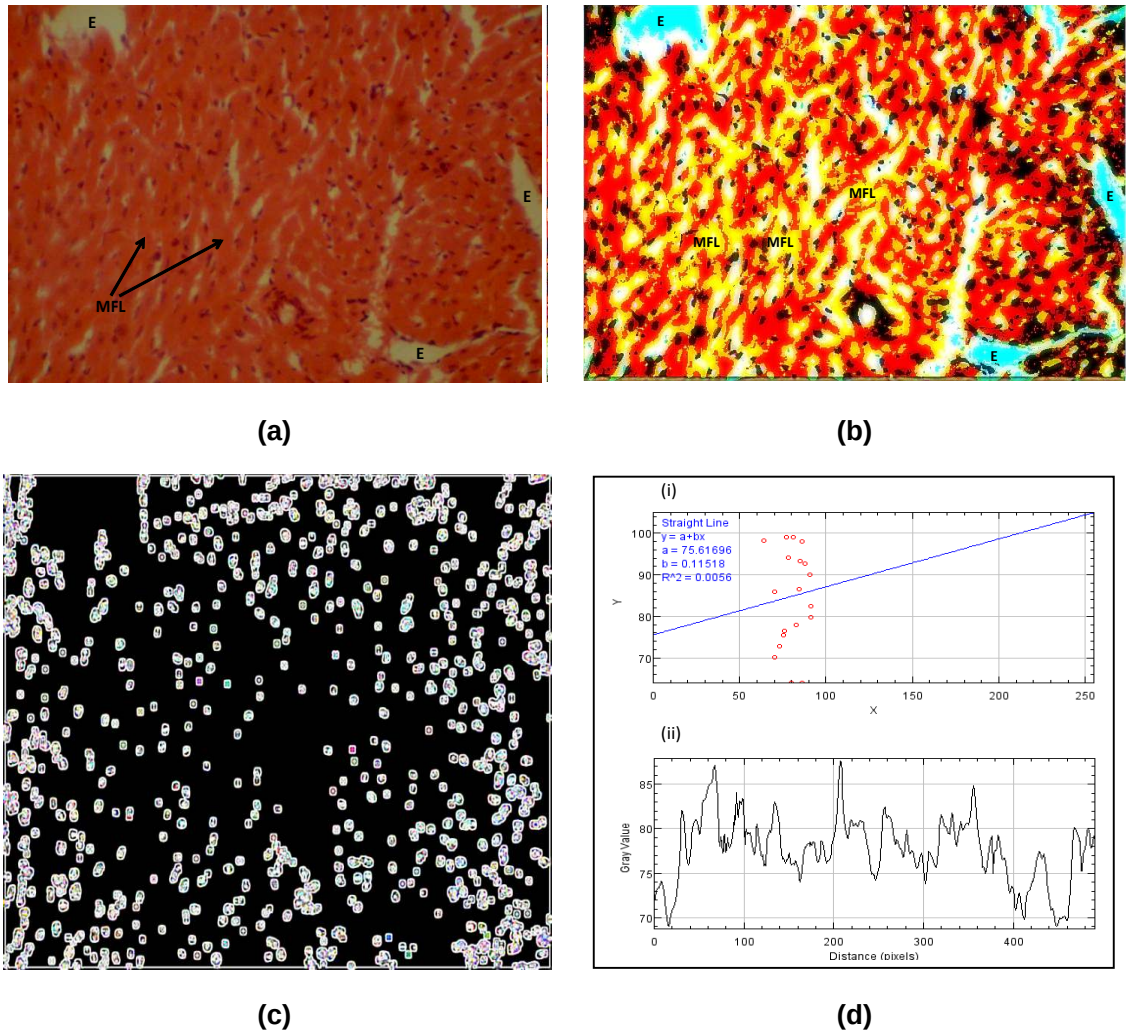

Fig. 2. (a) Photomicrograph of the transverse section of the heart of group IIA treated rat, administered with lower dose (200 mg/kg) of developer effluent for a short term period of 14 days (H and E Stains x400); (b) Representation of the image color correction and improvement application; (c) Representative image of an analyzed field showing threshold color application; (d) Graphical representation of the cardiac histology (i) straight line plot showing the calibration of the image intensities within the region of interest (ii) A profile plot of particles within the region of interest. E = edema (mild), MFL = myocardial fiber loss (occassional)

The cardiac histology of group IIIA (administered with 400 mg/kg of effluent for 14 days) indicated destruction and necrosis of the intercalated discs; degenerated, distorted and enlarged myocardial morphology and moderate cardiac edema compared to the control group (Fig. 3). Results of the image analysis (Table 1) indicated that group IIIA had greater particle count (n = 826), greater number of nuclei (n = 198) but lower CT spaces (n = 9) compared to the control (particle count, n = 452; nuclei, n = 185; CT spaces, n = 21). Other data of the image analysis are as summarized in the table.

There was severe degeneration, distortion and enlargement of myocardial morphology, with loss of intercalated discs as well as enlargement and separation of the connective tissues in the cardiac histology of group IIB (administered with 200 mg/kg of effluent for 28 days). However, the nuclei indicated no change when compared with the control group (Fig. 4). Results of the image analysis (Table 1) indicated that group IIB had greater particle count (n = 739), greater number of nuclei (n = 243) but lower number of CT spaces (n = 8) compared to the control (particle count, n = 452; nuclei, n = 185; CT spaces, n = 21). Other data of the image analysis are as summarized in the table.

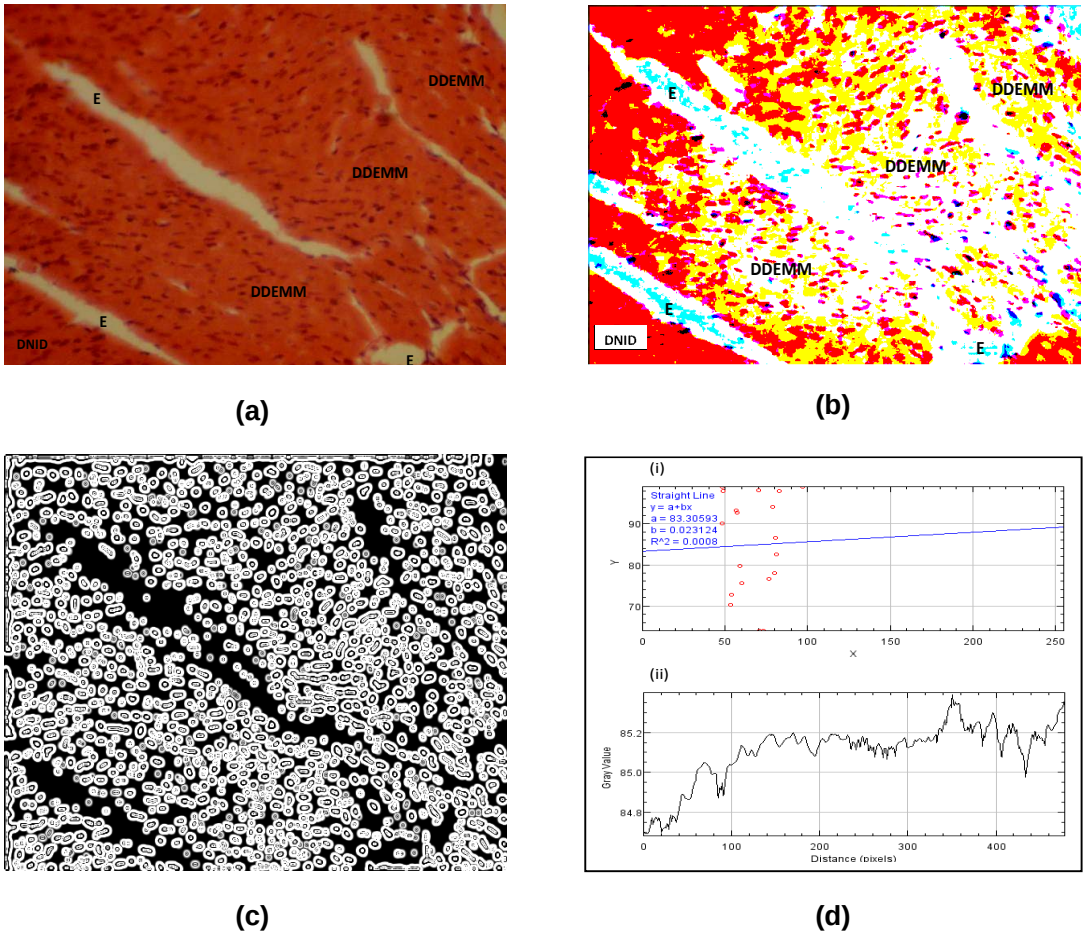

Fig. 3. (a) Photomicrograph of the transverse section of the heart of group IIIA treated rat, administered with 400 mg/kg of developer effluent for a period of 14 days (H and E Stains x400); (b) Representation of the image color correction and improvement application; (c) Representative image of an analyzed field showing threshold color application; (d) Graphical representation of the cardiac histology (i) straight line plot showing the calibration of the image intensities within the region of interest (ii) A profile plot of particles within the region of interest. DDEMM = Degenerated, distorted and enlarged myocardial morphology; DNID = Destroyed and necrotic intercalated disc; E = Edema

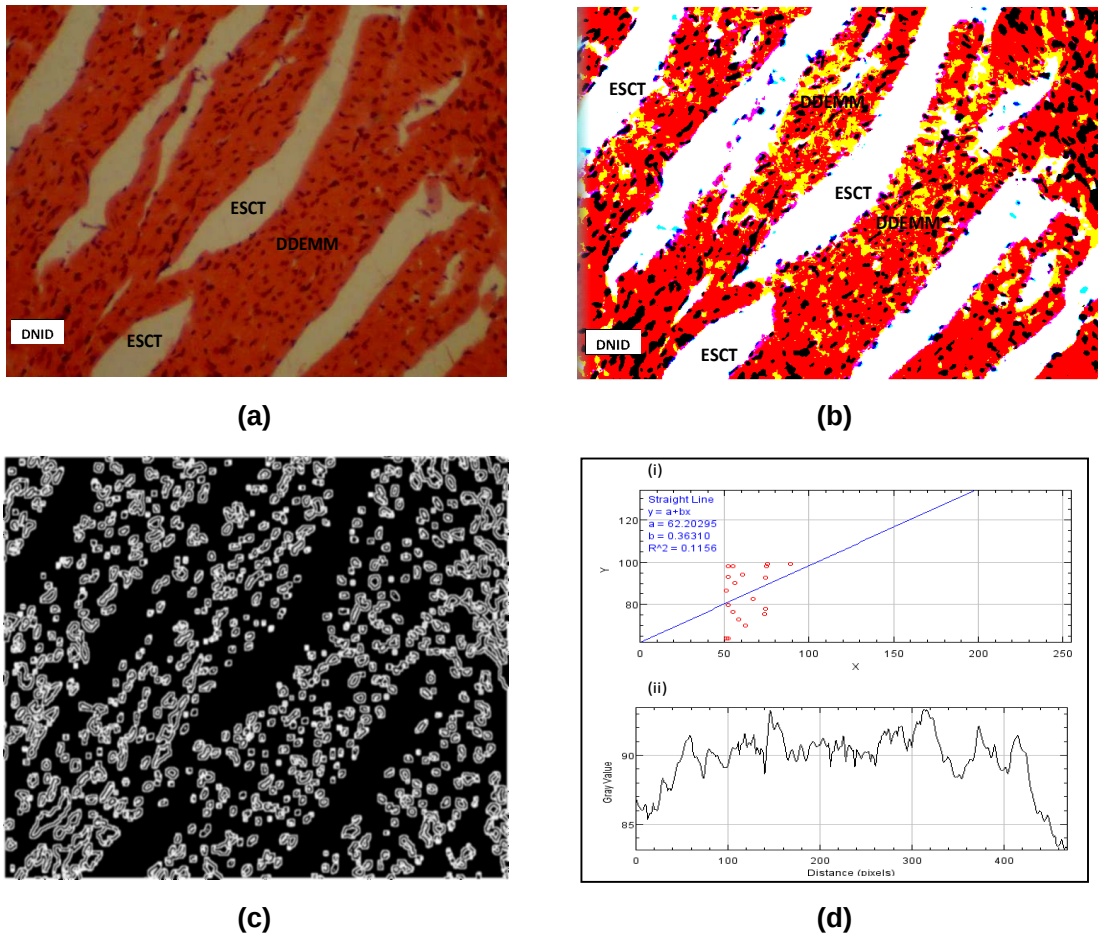

Fig. 4. Photomicrograph of the transverse section of the heart of group IIB treated rat, administered with 200mg/kg of developer effluent for a period of 28 days (H and E Stains x400); (b) Representation of the image color correction and improvement application; (c) Representative image of an analyzed field showing threshold color application; (d) Graphical representation of the cardiac histology (i) straight line plot showing the calibration of the image intensities within the region of interest (ii) A profile plot of particles within the region of interest. DDEMM = Degenerated, distorted and enlarged myocardial morphology; DLID = Destruction and loss of intercalated disk; ESCT = Enlarged and separated connective tissue

Table 1. Results of image analysis of the cardiac histology in control and test groups

Characteristics	Control (Fig. 1)	Group IIA (Fig. 2)	Group IIIA (Fig. 3)	Group IIB (Fig. 4)	Group IIIB (Fig. 5)
Image color threshold	52 – 255	129 – 255	129 – 255	132 – 255	135 – 255
Total surface area of ROI (µ)	613, 263	625,575	545,911	583,907	581,151
Average size of ROI (µ)	6,524.07	18,399.26	4,587.48	16,683.05	13,515.14
Mean gray value within ROI	25.51	73.20	61.39	60.75	55.21
ID gray value within ROI (µ)	238,836	1,366,833	357,989	1,276,790	981,594
Particle count within ROI	452	839	826	739	1103
% Area of particles within ROI	85.86	87.35	76.23	81.53	81.15
Circularity of particles	0.929	0.827	0.818	0.864	0.844
Number of nuclei	185	490	198	243	229
Number of CT spaces	21	43	9	8	8

Abbreviations: ROI = Region of Interest; ID = Integrated Density; CT = Connective Tissue

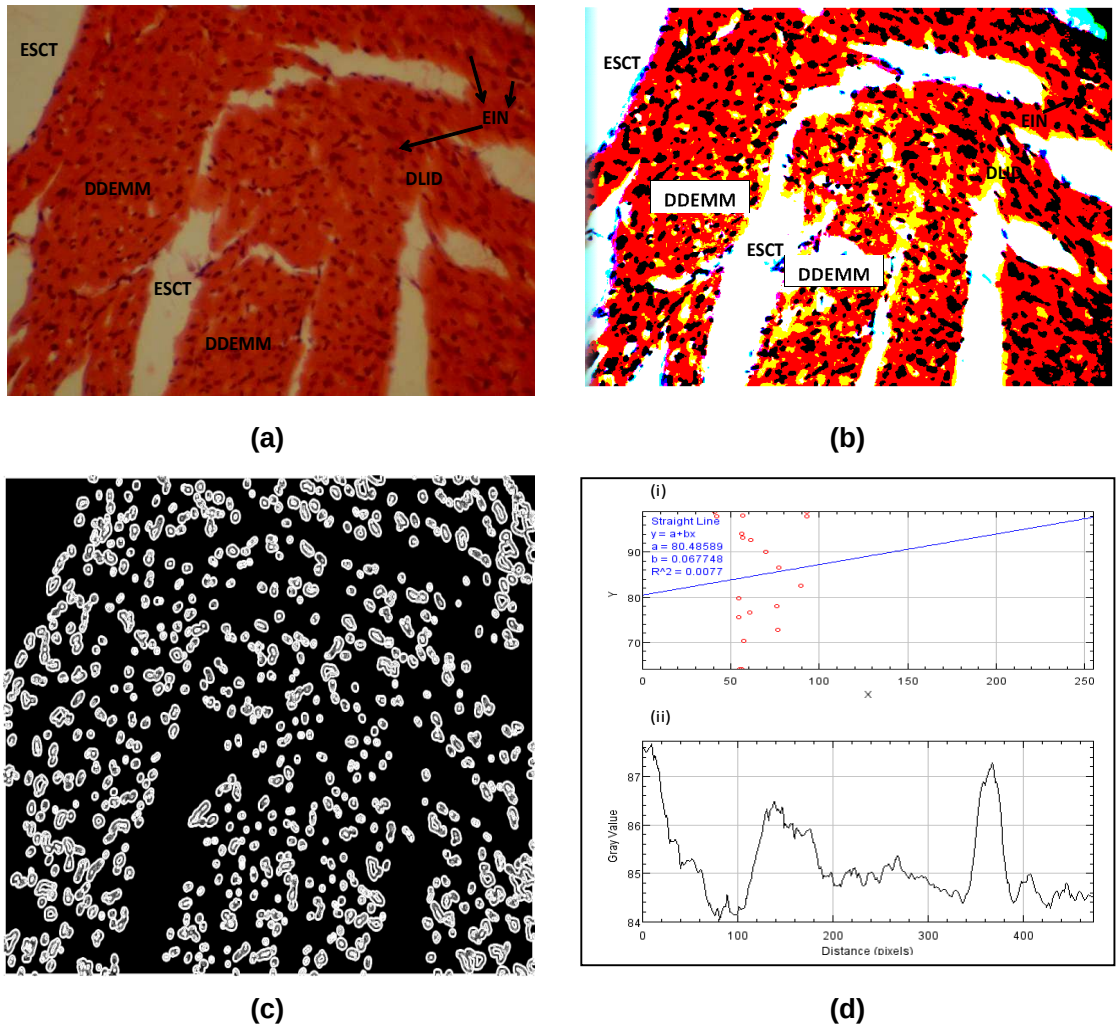

Fig. 5. (a) Photomicrograph of the transverse section of the heart of group IIIB treated rat, administered with 400 mg/kg of developer effluent for a period of 28 days (H and E Stains x400); (b) Representation of the image color correction and improvement application; (c) Representative image of an analyzed field showing threshold color application; (d) Graphical representation of the cardiac histology (i) straight line plot showing the calibration of the image intensities within the region of interest (ii) A profile plot of particles within the region of interest. DDEMM = Degenerated, distorted and enlarged myocardial morphology; DLID = Destruction and loss of intercalated disk; EIN = Enlarged and irregular nuclei; ESCT = Enlarged and separated connective tissue

There was loss of intercalated discs as well as enlargement and separation of connective tissues and degeneration, distortion and enlargement of myocardial morphology in group IIIB (administered with 400 mg/kg of effluent for 28 days). The nuclei were irregular and larger compared to the control group (Fig. 5). Results of the image analysis (Table 1) indicated that group IIIB had greater particle count (n = 1103), greater number of nuclei (n = 229) but lower number of connective tissue spaces (n = 8) compared to the control (particle count, n = 452; nuclei, n = 185; CT spaces, n = 21). Other data of the image analysis are as summarized in Table 1.

4. DISCUSSION

The principal findings of the present study revealed significant alterations in the histological profile of cardiac tissues of the experimental Wistar rat groups. Radiographic developer effluent given at a lower dose (200 mg/kg) for

14 days (group IIA) caused mild edema on the cardiac tissues and occasional loss of myocardial fibers. Developer administered for 14 days at higher dose of 400 mg/kg (group IIIA) showed alteration in the muscle fibers and necrosis of intercalated disk; there was also moderate distortion and enlargement of cardiac muscle structure and moderate cardiac edema when compared with control group. Long term (28 days) administration of lower dose (200 mg/kg) developer effluent (group IIB) caused severe distortion and enlargement of the cardiac muscle structure with destruction and loss of intercalated disk. Long term (28 days) administration of higher dose (400 mg/kg) developer effluent (group IIIB) caused severe distortion and enlargement of the cardiac muscle structure, destruction and loss of intercalated disks and irregular nucleus.

Certain conditions that affect osmotic pressure, such as hypotonic fluid overload, which allows the movement of water into the intracellular space, can cause edema [9]. The hypotonic content of the developer efflux may have allowed the passage and overloading of fluid through the syncytium of the cardiac cells thus causing the dose-dependent edema effects observed in the cardiac tissues of the groups IIA and IIIA respectively. The alterations and loss of the normal parallel alignment of myocytes is associated with myocardial fibrosis (the replacement of the myocytes with non-contractile scar tissue) [9]. The mechanism for the loss of myocardial fibers and edema in the present study is not clear. However, the myocardial disarray or dystrophy may be due to the presence of hydroquinone which has been reported to cause dystrophyic changes in myocardium [10,11].

Enlarged heart, also known as cardiomegally is a medical condition in which the heart is enlarged. [12] However, it is not a disease, but rather a symptom of another condition which may be caused by a large number of factors including toxins [12]. It is usually characterized by reduced overlap of the protein filaments actin and myosin within the sarcomeres of muscle fibers, thus impacting the heart's sliding filament mechanism. Thus cardiomegally may constitute one of the initial compensatory responses after an acute injury to cardiac contractile function. This response may help to transiently normalize the biomechanical stress and optimize cardiac pump function [13]. Previous reports have shown that cardiomegally is associated with the risk of congestive heart failure and sudden cardiac death [14,15] Intracellular accumulation of toxic substances has been implicated in the development of cardiac toxicity leading to cardiomyopathy and subsequent heart failure [16]. Similarly, clinical findings of heart failure are manifested when the mechanical dysfunction of the heart due to the toxic substance leads to hemodynamic compromise [16]. In the present study, the hypertrophy of the cardiomyocytes appeared to be dose-dependent as well as time-dependent and was evidenced by tortuous intercalated disk and nuclear irregularities. Cardiac hypertrophy has been previously associated with the presence of tortuous intercalated discs and nuclear irregularities [17].

Intercalated discs are microscopic identifying features of cardiac muscle. They connect individual heart muscle cells and enable them to work as a single functional organ or syncytium by supporting synchronized contraction of the cardiac tissue. Previous studies have demonstrated that cardiac defects and cardiomyopathies can be caused by the disruption of various intercalated disk components [18,19,20]. Toxic insults trigger series of reactions in cardiac cells leading to measurable changes. Mild injuries can be repaired, while cell death would occur from severe injuries through apoptosis or necrosis [16]. The disruption, loss or death of cardiac cells including intercalated discs is a part of myocardial injury that initiates or aggravates cardiomyopathy [16].

Tissue toxicity may be due to hemodynamic changes, direct actions of toxins on cells and tissue as well as immunological reactions due to inflammatory tissue injury [21]. It is not clear whether the histopathological changes observed in the heart tissues of rats in the present study are due to direct toxic effect of the constituents of the developer effluent or immunological reactions resulting from the tissue injury. Interestingly, a recent study has found that hydroquinone, a component of developer effluent, was able to enhance radical generation in RAW264.7 cells [22], suggesting its role as a strong pro-oxidant agent with chemical reactivity [23]. There has been some evidence to suggest that free radicals trigger and increase cell death mechanisms within the body such as apoptosis and in extreme cases necrosis [24]. It is possible that the cellular toxicity due to developer effluent overload may have generated free radicals which eventually caused cardiac tissue damage. In addition, hydroquinone, a major component of cigarette

smoke, has been implicated in increased rates of higher respiratory tract infection in chronic cigarette smokers [25] and reported to play a role in various immunotoxicological conditions [22]. Furthermore, exposure to sub-acute boric acid, another component of developer effluent, has been reported to cause dose-dependent histopathological degenerative changes in body tissues such as the kidneys [26].

5. LIMITATIONS OF STUDY

Cellular or tissue toxicity can be a result of direct actions of toxic chemicals or immunological reactions due to inflammatory tissue injury. One of the limitations of this study is that we could not carry out further investigations to elucidate the exact mechanisms behind the observed cardiotoxic effects of developer effluent on the Wistar rats. Our major focus was to ascertain if there were morphological alterations or histopathological changes in the cardiac tissues of the rat due to acute or chronic developer effluent exposures. Interestingly, the present study was able to elucidate some histopathological changes in the Wistar rats' cardiac architecture using a scientific multidimentional image processing and analytical software. However, in view of the above mentioned limitation, we do recommend further studies to explain the exact mechanisms behind the observed histopathological changes.

6. CONCLUSION

The present study indicated that both acute / chronic and long-term / short-term exposures to sub-lethal doses of developer effluent caused alterations in the histology of the heart of Wistar rats. The cardio-toxic effects observed include: loss of myocardial fibers, edema, destruction of intercalated discs of the heart and severe hypertrophy of cardiac myocytes. These effects could lead to cardiomyopathy, thus the need for proper management and disposal of developer effluents.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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