

## Original Research Articles

# CARDIOTOXIC ASSESSMENT OF RADIOGRAPHIC DEVELOPER EFFLUENT IN WISTAR RATS

## 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.

**Methodology:** Eighteen young Wistar rats of weights 140-220g were divided into three groups. The control group was sub-divided into two groups of 3 rats each and orally administered with 1ml of distilled water daily for 14 days and 28 days respectively; each of the experimental groups II and III were also sub-divided into two groups of three rats each administered with lower dose (200mg/kg) and higher dose (400mg/kg) of developer effluent daily for 14 days and 28 days respectively.

**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, while the higher dose caused severe distortion and enlargement of the cardio-myocytes, loss of intercalated discs, and irregular nucleus.

**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.

**Key words:** 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<sup>1</sup>. After its decomposition, developer produces substances such as carbon monoxide, carbon dioxide and oxides of sulphur, which are hazardous<sup>1</sup>. 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 <sup>2</sup>.

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 <sup>1</sup>. **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 <sup>3</sup>. 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 <sup>2, 4</sup>. Exposure to harmful and toxic substances is known to likely occur through the diet, from medications, the environment and workplace <sup>5</sup>. 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°C 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 <sup>6</sup>. 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 pH of 10.4. The lethal dose (LD<sub>50</sub>) concentration of the developer effluent was calculated as 2450 mg/kg body weight using the formula: LD<sub>50</sub> =  $\sqrt{a \times b}$  (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 <sup>7</sup>. The concentrations of the developer effluent used for the experiment were sub-lethal doses of 200mg/kg (lower dose) and 400mg/kg (higher dose) of body weight.

### 2.3 Experimental Design

The animals were divided randomly into three groups of 6 rats each - group I (control) and experimental groups II and III respectively. Group I was further divided into two groups of 3 rats each and administered with 1ml of distilled water for 14 and 28 days respectively. The other groups II, III which served as the experimental groups were administered with 200mg/kg and 400mg/kg of developer effluent respectively by oral gavage. The experimental groups II and III were further sub-divided into two groups (i.e. groups IIA and IIB and groups IIIA and IIIB) of three rats each. Thus group IIA rats were administered with low dose (200mg/kg) of fixer effluent for a short term period of 14 days; the group IIB rats were administered with the higher dose (400mg/kg) of fixer effluent for a short term period of 14 days; group IIIA rats were administered with low dose (200mg/kg) for long term period of 28 days; and group IIIB rats were administered with higher dose (400mg/kg) for long term period of 28 days. The average developer effluent consumption was 0.2 ml/day for the low dose (200mg/kg) group and 0.42ml for the high dose (400mg/kg) group. The present experiment was designed to be time and dose-dependent with the same low dose (200 mg/kg or 400mg/kg) being administered daily to Group II or III for 14 days and 28 days respectively. After 14 days, three rats from groups I, II and III were sacrificed (using the chloroform inhalation method), while the remaining three rats in each of the groups 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, labelled 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.

### 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)<sup>8</sup>. 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.

## 3. RESULTS

Figure 1 shows the normal micro-architecture of the heart. The cardiac histology of the control rats revealed a normal appearance showing normal and centrally arranged nucleus, the intercalated disks and connective tissues also appeared normal and the cardiac muscle fibers are well arranged.

Figure 2 shows the photomicrograph of the heart of group IIA treated with low dose developer effluent for 14 days. The cardiac histology revealed significant alterations in the histological profile when compared to the control group. The histological findings include presence of mild edema and occasional loss of myocardial fibers.

Figure 3 shows the photomicrograph of the heart tissue of group IIB rats treated with high dose developer effluent after 14 days. The cardiac histology indicated destruction and necrosis of the intercalated disk; degenerated, distorted and enlarged myocardial morphology and moderate cardiac edema compared to the control group.

Figure 4 shows the photomicrograph of the heart tissue of group IIIA treated with low dose developer effluent after 28 days. There was severe degenerated, distorted and enlarged myocardial morphology, with loss of intercalated disk and enlargement and separation of the connective tissues. However, the nuclei indicated no change when compared with the control group.

Figure 5 shows the photomicrograph of a section of the heart tissue of group IIIB treated with high dose developer effluent after 28 days. There was loss of intercalated disks and enlargement and separation of the connective tissues as well as degeneration, distortion and enlargement of myocardial morphology. The nuclei were irregular and larger compared to the control group.

#### 4. DISCUSSION

The principal findings of the present study indicate that radiographic developer effluent given at a lower dose (200 mg/kg) for two weeks (group IIA) caused mild edema on the cardiac tissues and occasional loss of myocardial fibers. Developer administered for two weeks at high dose of 400 mg/kg (group IIB) 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 low dose (200mg/kg) developer effluent (group IIIA) caused severe distortion and enlargement of the cardiac muscle structure with destruction and loss of intercalated disk. Long term (28 days) administration of high dose (400mg/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<sup>9</sup>. 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 IIB respectively. The alterations and loss of the normal parallel alignment of myocytes (the muscle cells of the heart) is associated with myocardial fibrosis (the replacement of the myocytes with non-contractile scar tissue)<sup>9</sup>. The mechanism for the loss of myocardial fibres 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 dystrophic changes in myocardium<sup>10,11</sup>.

Enlarged heart, also known as cardiomegaly is a medical condition in which the heart is enlarged.<sup>12</sup> 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<sup>12</sup>. It is usually characterized by an increase in size of cardiomyocytes, accompanied by reduced overlap of the protein filaments actin and myosin within the sarcomeres of muscle fibers, thus impacting the heart's sliding filament mechanism (<https://en.wikipedia.org/wiki/Cardiomegaly>). It may initially constitute one of the 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<sup>13</sup>. Previous reports have shown that cardiomegaly is associated with the risk of congestive heart failure and sudden cardiac death<sup>14,15</sup>. Intracellular accumulation of toxic substances has been implicated in the development of cardiac toxicity leading to cardiomyopathy and subsequent heart failure<sup>16</sup>. Similarly, clinical findings of heart failure are manifested when the mechanical dysfunction of the heart due to the toxic substance leads to hemodynamic compromise<sup>16</sup>. In the present study, the hypertrophy of the cardiomyocytes appeared 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<sup>17</sup>.

Intercalated discs are microscopic identifying features of cardiac muscle. They connect individual heart muscle cells (cardiomyocytes) and enable them to work as a single functional organ or syncytium by supporting synchronised 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<sup>18, 19, 20</sup>. 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<sup>16</sup>. The disruption, loss or death of cardiac cells including intercalated discs is a part of myocardial injury that initiates or aggravates cardiomyopathy<sup>16</sup>.

Tissue toxicity may be due to hemodynamic changes, direct actions of toxins on cells and tissue and immunological reactions due to inflammatory tissue injury<sup>21</sup>. It is not clear whether the histopathological changes observed the heart tissues of the rats in the present study may be a 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<sup>22</sup>, suggesting its role as a strong pro-oxidant agent with chemical reactivity<sup>23</sup>. 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<sup>24</sup>. 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 the increased rates of higher respiratory tract infection in chronic cigarette smokers<sup>25</sup> and reported to play a role in various immunotoxicological conditions<sup>22</sup>. 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<sup>26</sup>.

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. However, 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 in this study 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. In view of the above limitation, we therefore recommend further studies to elucidate these facts.

## 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.

## Conflict of Interest

“The authors declare that there are no conflicts of interest”.

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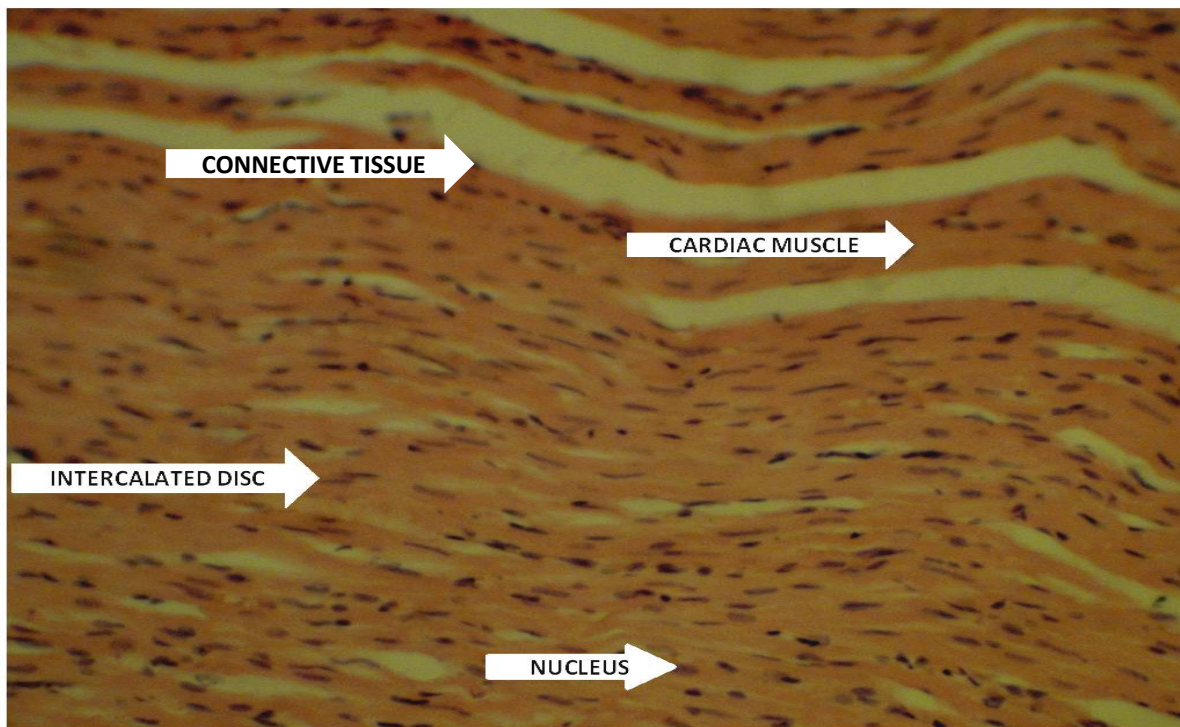
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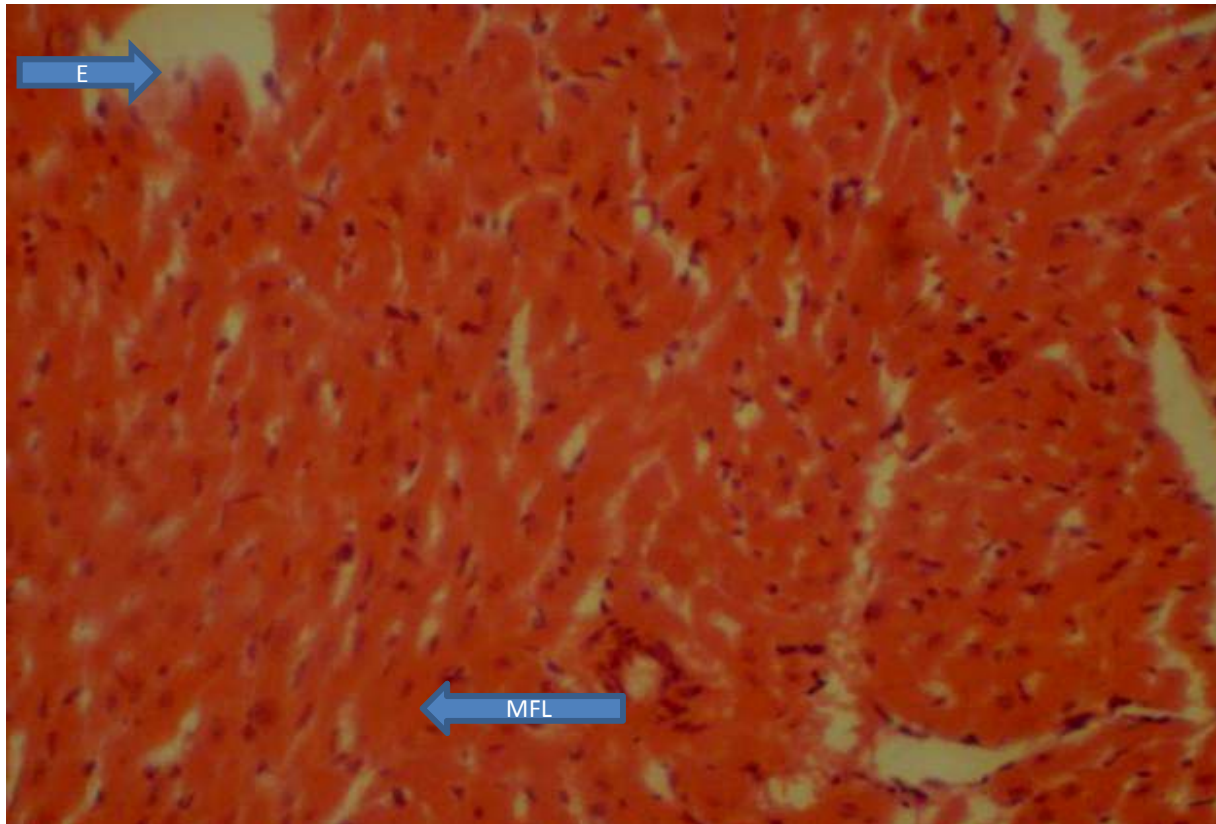
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**Figure 1:** Photomicrograph showing normal architecture of the heart of group I (control) rat. H and E Stain x400. The nuclei, the connective tissues and cardiac muscle fibers appeared normal and well arranged.

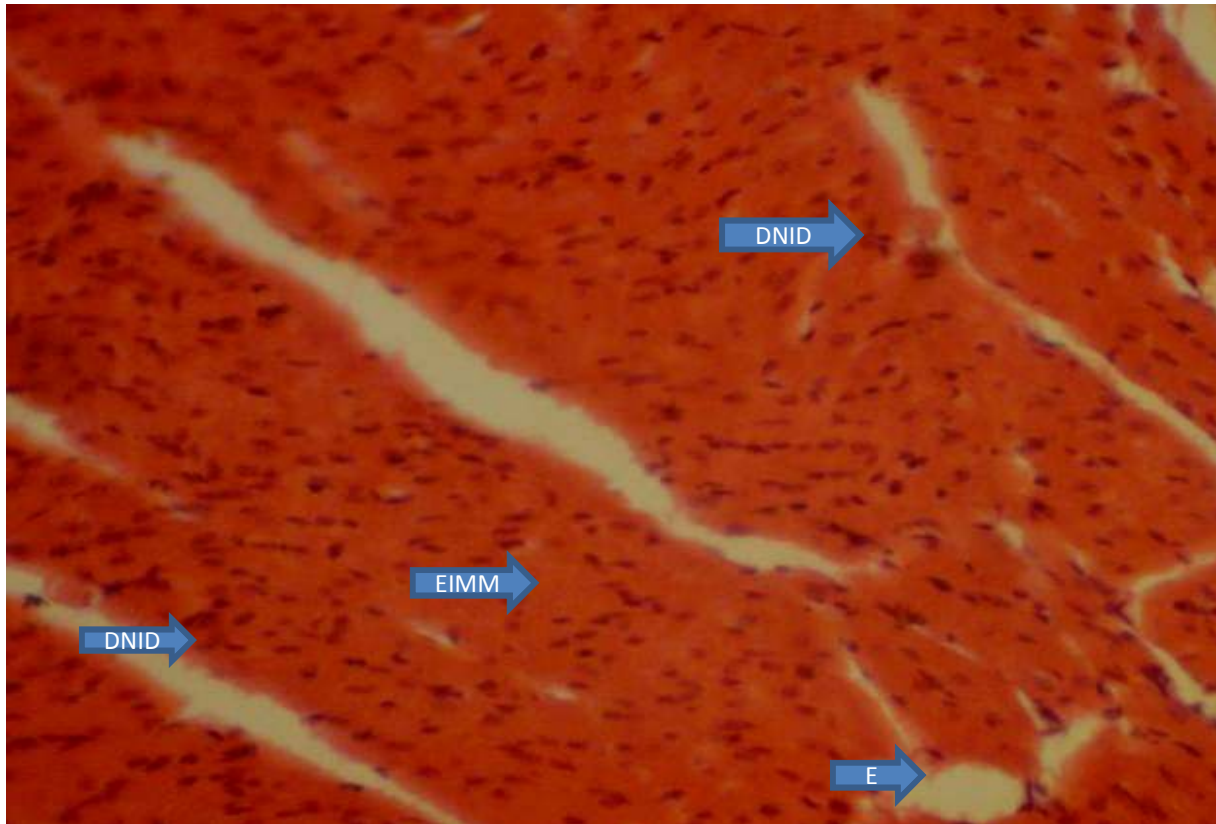
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**Figure 2:** Photomicrograph of the transverse section of the heart of group IIA treated rat, administered with low dose (200mg/kg) of fixer effluent for a short term period of 14 days, (H and E Stains x400). E = edema (mild), MFL = myocardial fiber loss (occasional)

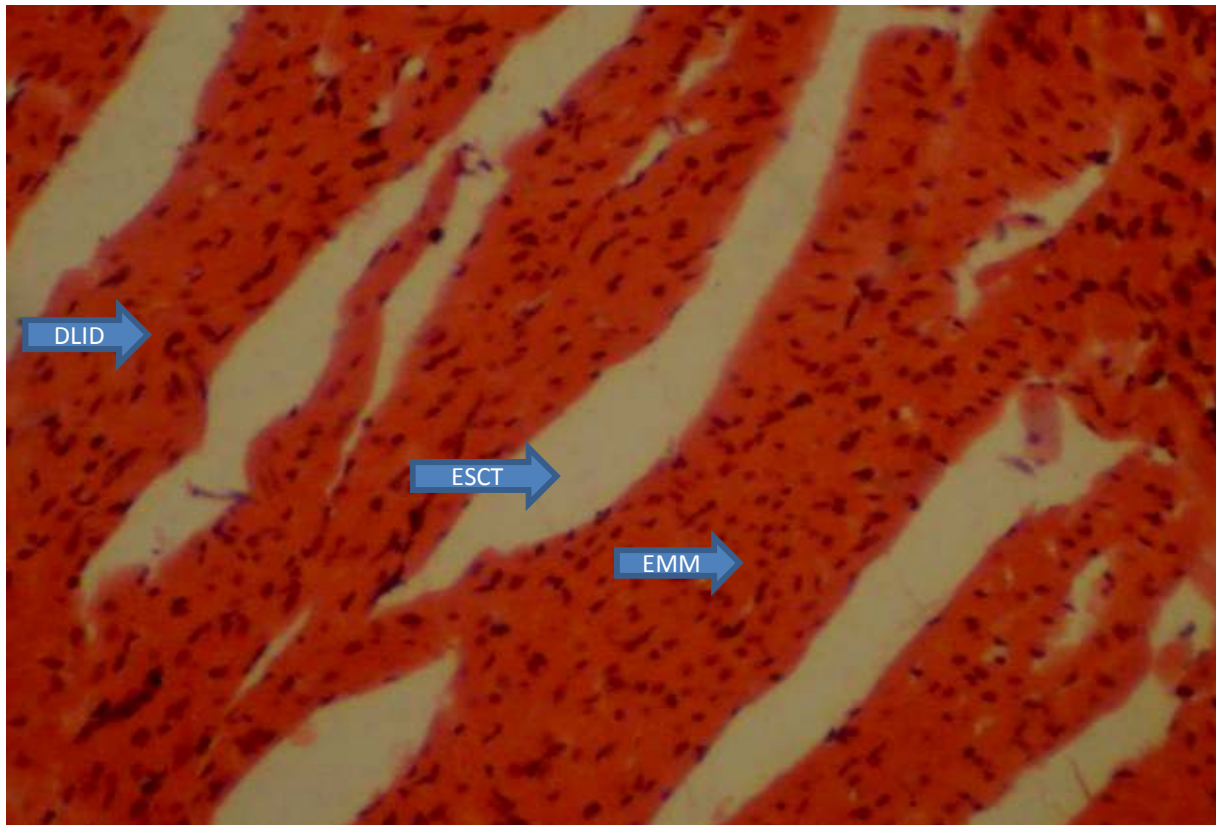
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**Figure 3:** Photomicrograph of the transverse section of the heart of group IIB treated rat, administered with 400mg/kg of fixer effluent for a period of 14 days, (H and E Stains x400). DNID = Destroyed and necrotic intercalated disc; EIMM = degenerated, distorted and enlarged myocardial morphology; E = edema.

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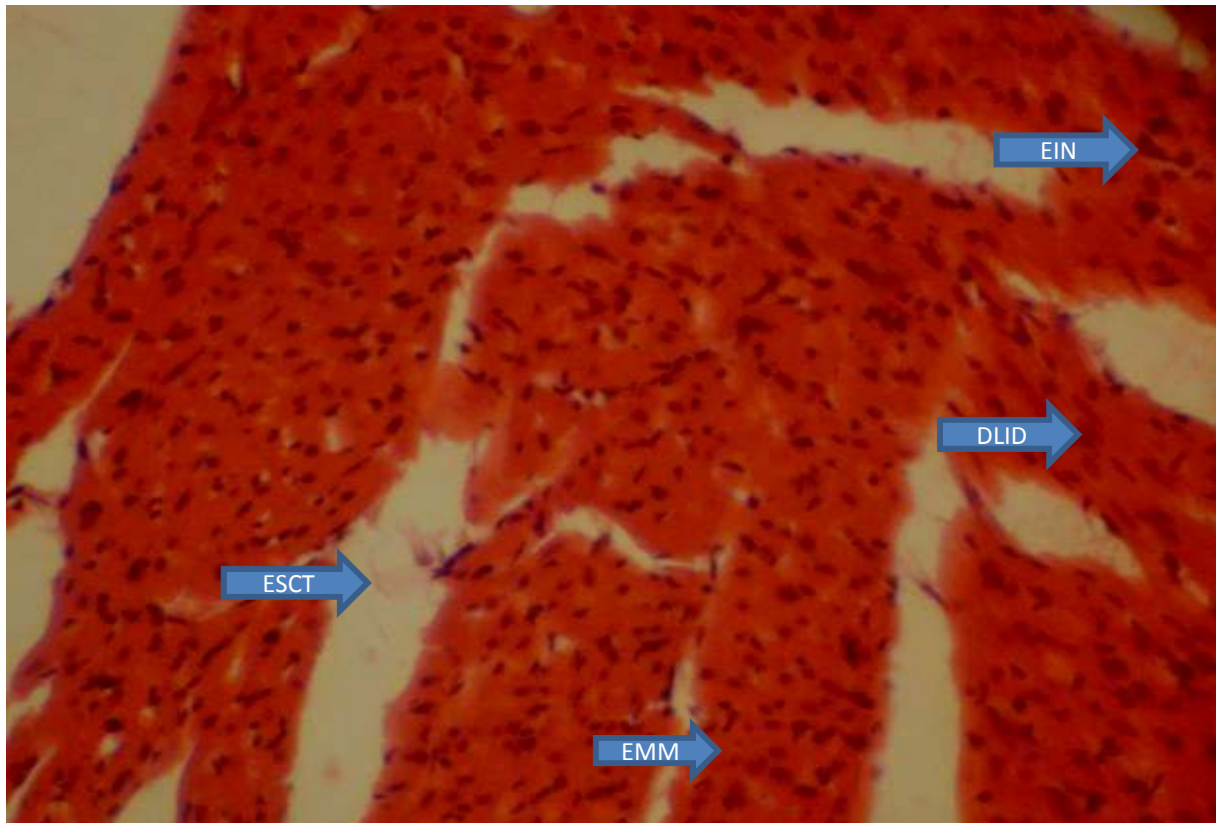
**Figure 4:** Photomicrograph of the transverse section of the heart of group IIIA treated rat, administered with 200mg/kg of fixer effluent for a period of 28 days, (H and E Stains x400). EMM = degenerated, distorted and enlarged myocardial morphology; DLID = Destruction and loss of intercalated disk; ESCT = Enlarged and separated connective tissue

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**Figure 5:** Photomicrograph of the transverse section of the heart of group IIIB treated rat, administered with 400mg/kg of fixer effluent for a period of 28 days, (H and E Stains x400). EMM = Enlarged myocardial morphology, DLID = Destruction and loss of intercalated disk; ESCT = Enlarged and separated connective tissue