

## **Original Research Articles**

### **CARDIOTOXICITY ASSESSMENT OF RADIOGRAPHIC DEVELOPER EFFLUENT IN WISTAR RATS**

#### **ABSTRACT**

**Background:** Cellular changes associated with toxicity of radiographic developer effluents on heart tissues have not been studied.

**Objectives:** We aimed at demonstrating the histopathological changes in cardiac tissues of wistar rats following exposure to developer effluent.

**Methods:** Twenty 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 hypertrophy of cardio-myocytes and edema. Administration of lower dose developer effluent for 28 days caused severe hypertrophy of the cardio-myocytes with destruction and loss of intercalated disk, while the higher dose caused severe hypertrophy of the cardio-myocytes, loss of intercalated discs, alterations of muscle fiber structure 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

#### **INTRODUCTION**

Radiographic developers are widely used in the photographic and X-ray film processing. It is reported to have harmful effects if inhaled or accidentally swallowed. It also liberates toxic gases which are irritating to the eyes, skin and the gastrointestinal tract as well as cause damage to the kidneys<sup>1</sup>. Little is known of other hazards of the chemical except that, on decomposition, it produces toxic substances such as hazardous carbon monoxide, carbon dioxide and oxides of sulphur<sup>1</sup>. Furthermore, the exhausted waste known as developer effluent 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>. In developed countries, there are clear legal rules that give guidance for the proper discharge of these effluents; 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 with levels of inorganic compounds above allowed limits, without previous treatment or recycling. Additionally, such effluents are discarded with a high chemical oxygen demand (COD) and hydrogenic potential (pH), and color, total dissolved solids concentration, chlorides, sulfates and turbidity over allowed limits<sup>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.

## MATERIALS AND METHODS

### 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.

### 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 (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<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.

### 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 orally administered with 200mg/kg and 400mg/kg of developer effluent respectively. The experimental groups II and III were further sub-divided into two groups of three rats each, which were orally administered with 200mg/kg and 400mg/kg of developer effluents daily for 14 days (groups IIA and IIB) and 28 days (groups IIIA and IIIB) respectively. 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.

#### 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.

#### 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.

## RESULTS

Figure 1 shows the normal micro-architecture of the heart, the muscle fibers (marked with the green arrow). The nuclei of the cardiac muscle cells lie in the middle of the cells (marked with the red arrow). The intercalated disk that connected one cardiac muscle to another was marked with white arrow. Each muscle fiber is surrounded by an endomysium of delicate connective tissue with a rich capillary network.

Figure 2 shows the photomicrograph of the heart of the treated group IIA with significant alteration in the histological profile when compared with that of the heart in the control group. The histological alteration seen in the sections of the heart in the treated group are mild edema and occasional loss of myocardial fibers.

Figure 3 shows the photomicrograph of the heart tissue of the rats treated with high dose developer effluent after 14 days (group IIB) having alterations in the muscle fibers architecture; destruction and necrosis of the intercalated disk; moderate hypertrophy of cardiac myocytes and moderate cardiac edema when compared with the control group.

Figure 4 shows the photomicrograph of the heart tissue treated with low dose developer effluent after 28 days (group IIIA). There was severe hypertrophy of cardiac muscles, with the loss of intercalated disk and the cardiac lumen becoming larger. However, the nucleus shows no change when compared with the control group.

Figure 5 shows the photomicrograph of a section of the heart tissue with alteration in the cardiac muscle structure. There was also loss of intercalated disks and a marked increase in the cardiac luminal diameter after 4 weeks at high dose (group IIIB). The nucleus was enlarged when compared with the control group.

## 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 hypertrophy of cardiac myocytes 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 hypertrophy of the cardiac myocytes with destruction and loss of intercalated disk. Long term (28 days) administration of high dose (400mg/kg) developer effluent (group IIIB) caused severe hypertrophy of the cardiac myocytes, destruction and loss of intercalated disks, alterations of muscle fiber structure and irregular nucleus.

Edema can be caused by a variety of factors, including conditions that affect osmotic pressure, such as hypotonic fluid overload, which allows the movement of water into the intracellular space<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>.

Cardiac hypertrophy is defined as a thickening of the interventricular wall and/or septum<sup>12</sup>; in the cell, it is characterized by an increment in cardiomyocyte size, with increased protein synthesis and changes in the organization of the sarcomeric structure.<sup>13</sup> After acute injury to cardiac contractile function, cardiac hypertrophy initially constitutes one of the compensatory responses that transiently normalizes the biomechanical stress and optimizes cardiac pump function<sup>14</sup>. However, prolonged hypertrophy is a highly important risk factor for the

development of heart failure.<sup>15,16</sup> Intracellular accumulation of toxic substances has been implicated in the development of cardiac toxicity leading to cardiomyopathy and subsequent heart failure<sup>17</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>17</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>18</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 demonstrate that the disruption of various intercalated disk components leads to cardiac defects and cardiomyopathies<sup>19, 20, 21</sup>. Toxic insults trigger series of reactions in cardiac cells leading to measurable changes. Mild injuries can be repaired; however, severe injuries will lead to cell death via apoptosis or necrosis<sup>17</sup>. The disruption, loss or death of cardiac cells including intercalated discs is a part of myocardial injury that initiates or aggravates cardiomyopathy<sup>17</sup>.

## Conclusion

The present study indicated that both acute/chronic and long-term/short-term exposures to sub-lethal doses of fixer 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 effluent.

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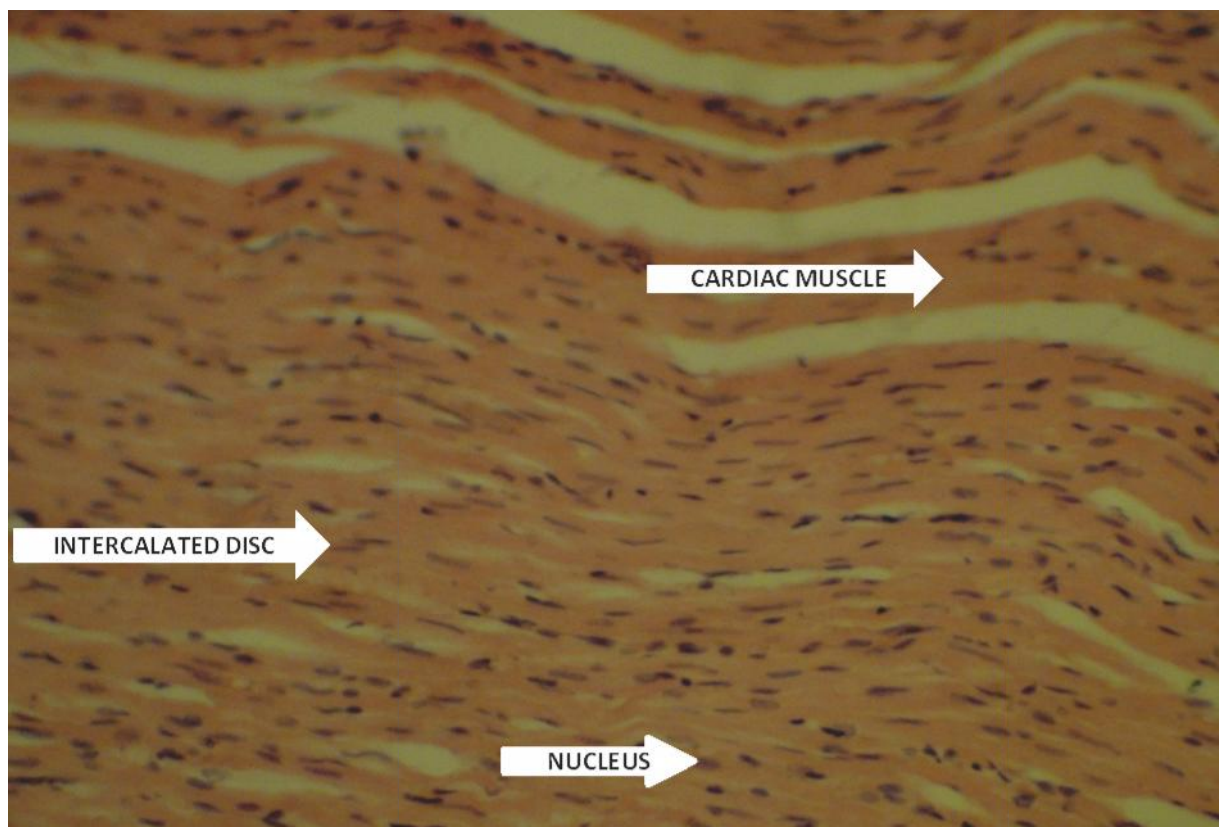


**Figure 1:** Photomicrograph of the heart of control administered with distilled water for 4 weeks. Stained by H&E (x400).

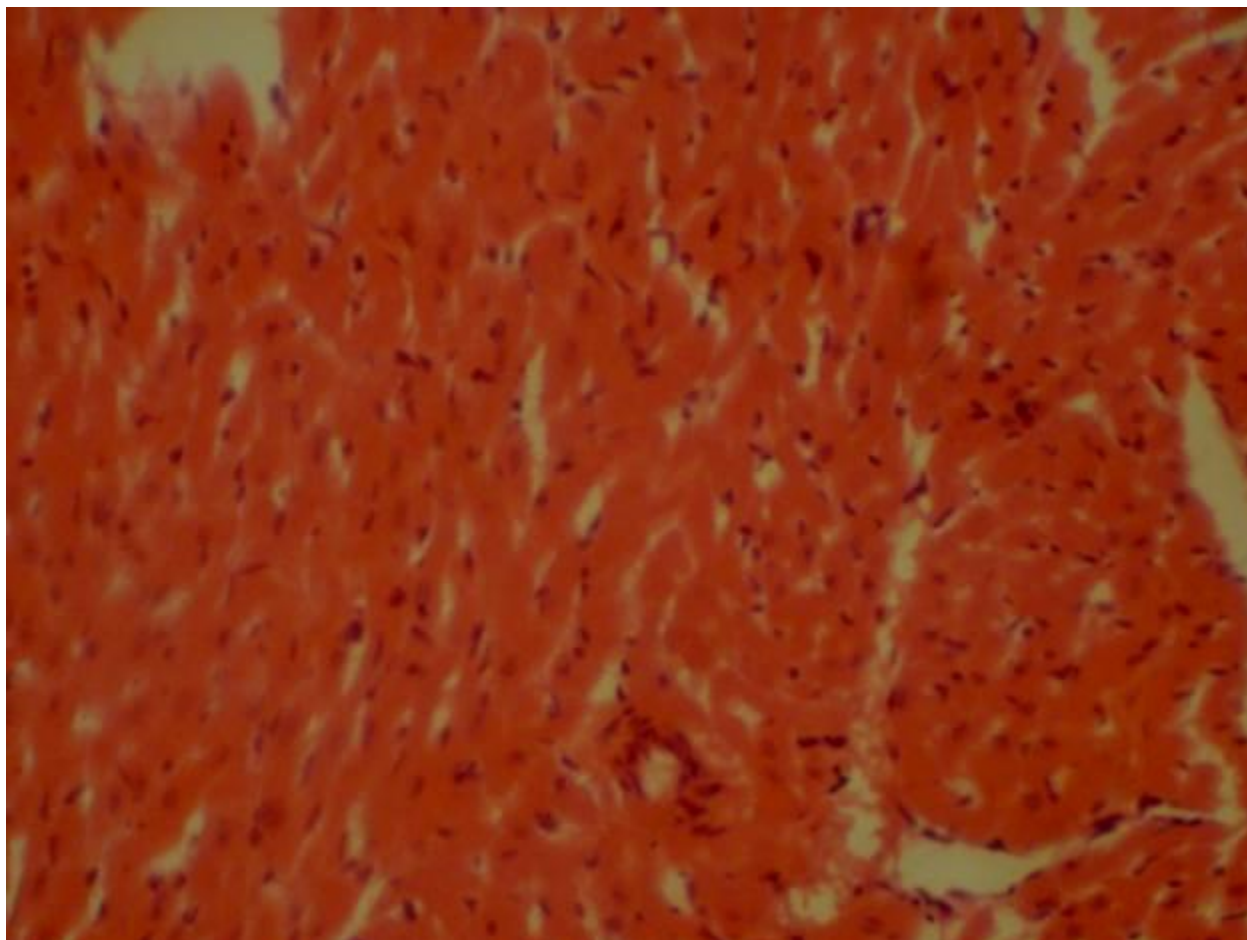


**Figure 2:** Photomicrograph of the heart of wistar rat showing the effect of short term oral administration of low dose developer effluent (group IIA) on the heart tissues. Stained by H&E (x400).

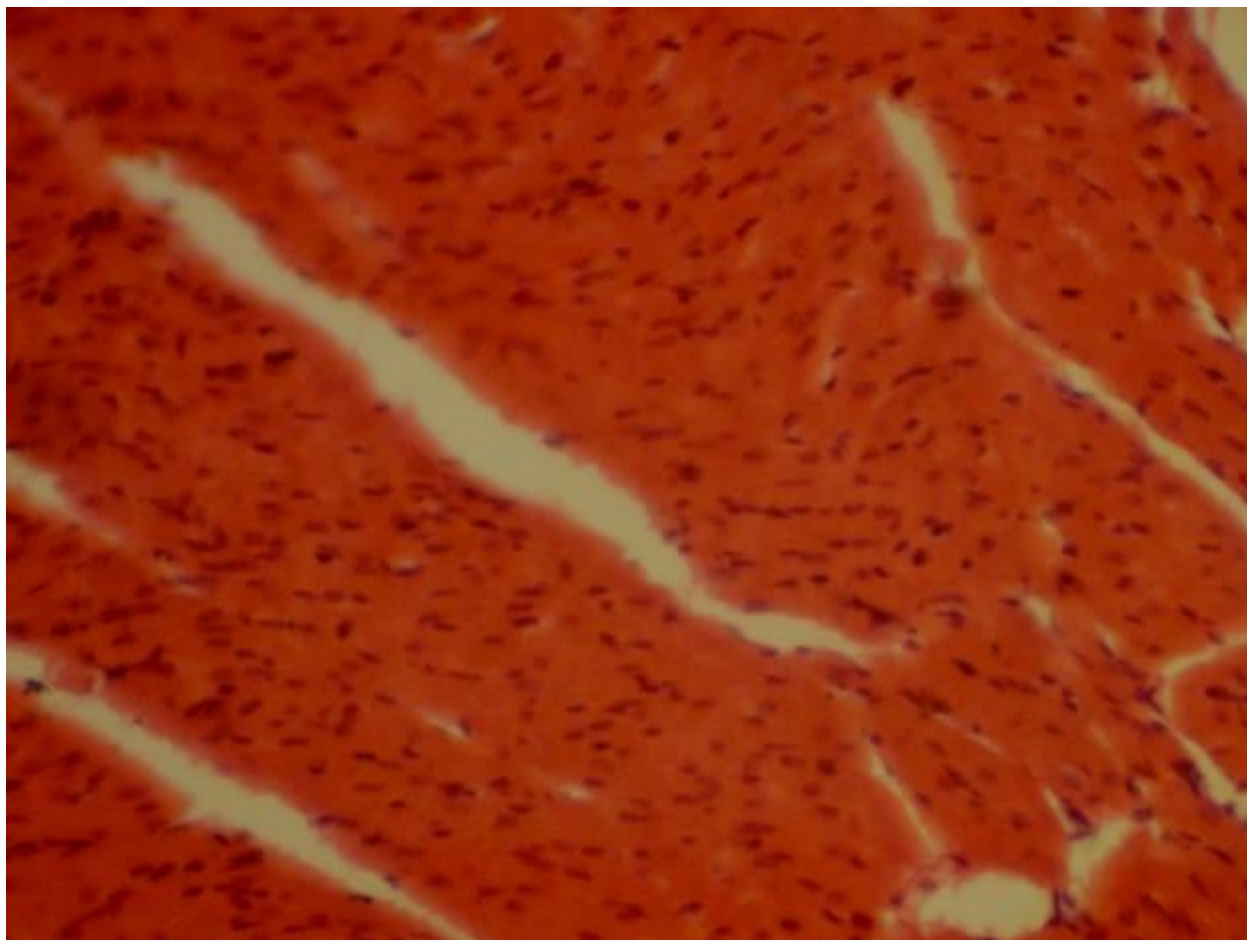
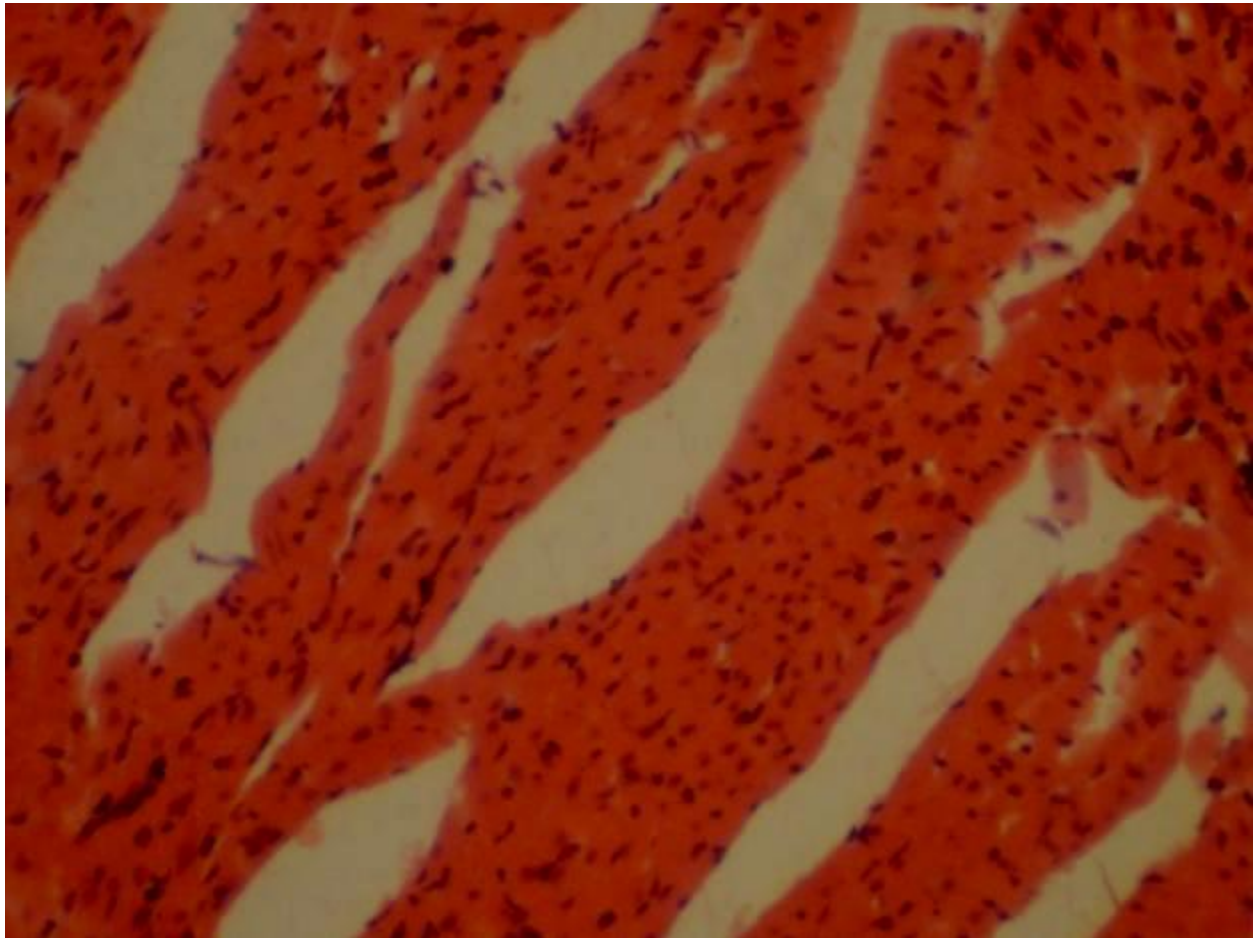


**Figure 3:** Photomicrograph of the heart of wistar rat showing the effect of short term oral administration of high dose developer effluent (group IIB) on the heart tissues. Stained by H&E (x400).

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296 **Figure 4:** Photomicrograph of the heart of wistar rat showing the effect of long term oral  
297 administration of low dose developer effluent (group IIIA). Stained by H&E (x400).

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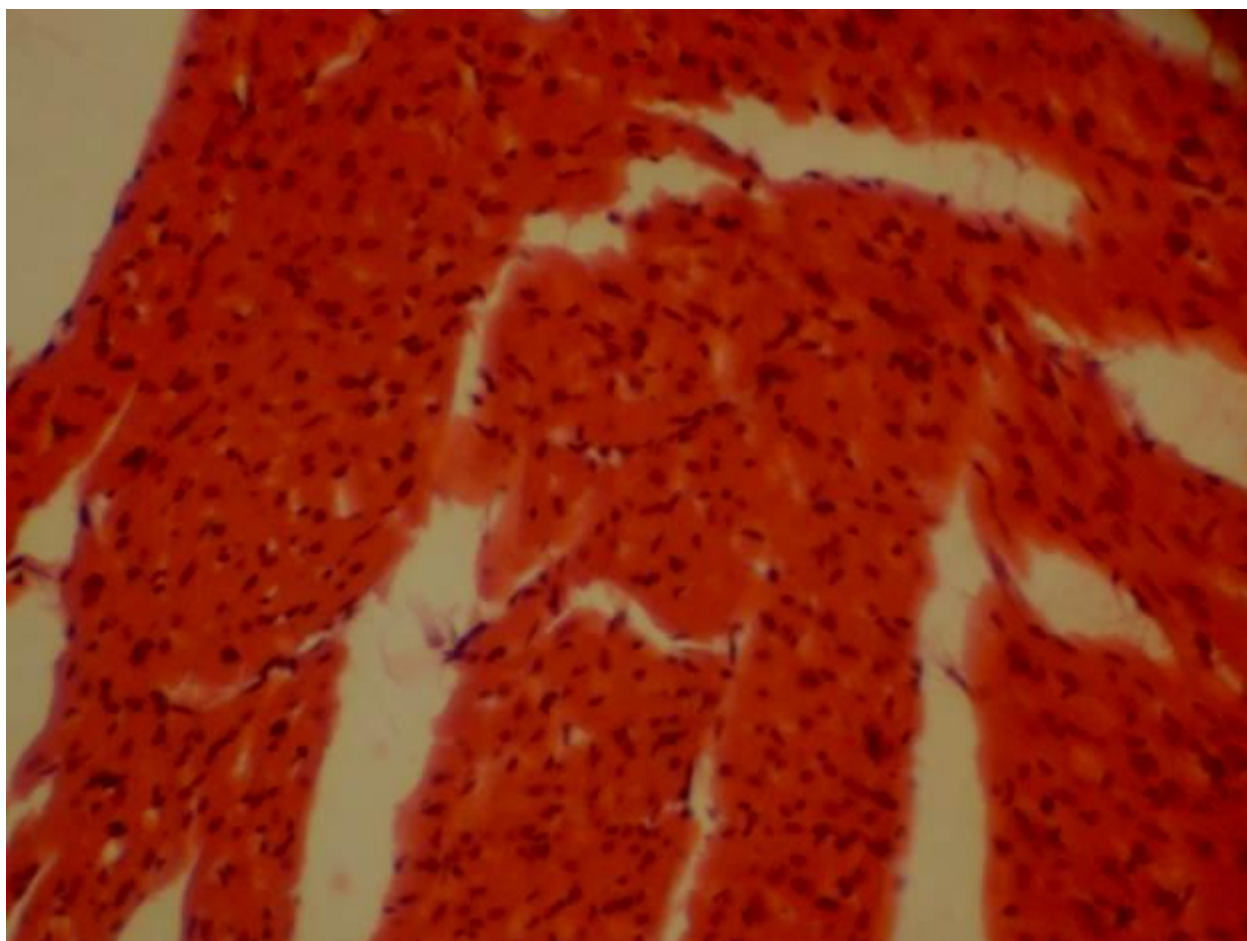
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309 **Figure 5:** Photomicrograph of the heart of wistar rat showing the effect of long term oral  
310 administration of high dose developer effluent (group IIIB). Stained by H&E (x400).

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