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EVALUATION OF MICRO-ARCHITECTURAL POSTMORTEM CHANGES OF CARDIAC TISSUE OF *SUS SCROFA DOMESTICA* FOR ASSESSMENT OF DECAY RATE AND ESTIMATION OF POSTMORTEM INTERVAL

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Abstract

Different tissues have peculiar decomposition science, and the histological study of such postmortem changes plays a vital role when carrying out prediction of postmortem interval. The present study was an attempt to evaluate the histological postmortem changes of cardiac tissues of *Sus Scrofa domesticus* for Forensic purposes. This study cohort involved sixteen animals weighing between 30 to 35kg. The research was carried out at the Department Anatomy and Forensic Anthropology Research Facility (DAFARF) UNICROSS, Okuku Campus. The sixteen pigs used pigs were euthanized using axillary sharp trauma. The organs of interest (heart) were eviscerated from the sixteen porcines and kept in the research facility under room condition to prevent canids from feasting on the organ. The study lasted for 5days using 0-102 hour's postmortem interval (PMI) and samples collected at various intervals (three times daily). Samples were preserved with bouin's fluid and transferred to 70% ethanol after 6 hours and routine tissue processing techniques employed. The samples were stained using Hematoxylin and Eosin (H&E), Periodic acid Schiff (PAS) and Masson's trichrome stains, respectively. The slides were examined using light microscope, it was observed that at 0-6 hours PMI, the cardiac tissues maintained normal microarchitecture (fresh stage) as compared to 10 hours PMI with disoriented architecture. Moderate effects were also observed in day 2 (morning, afternoon, and evening) which conforms to bloated stages of decay, as compared to the severe effects observed between day three (Active decay stage) to day five (advanced decay stage) respectively. Therefore, the changes observed in this study can be applied, and relied upon in forensic medico-legal investigations when determination of postmortem interval is sort for by forensic scientists.

1.0 Introduction

In forensic investigations, histological tests are commonly employed to determine the cause and manner of death [1]. Histology provides vital information on the cellular and structural alterations that take place in various tissues, which aids in the identification of pathological illnesses and injuries [1]. It is possible to assess the histological changes in heart tissue, which can be crucial in cases of cardiac-related deaths. However, interpretation may be challenging due to postmortem histological changes in cardiac tissue, which is why it is imperative to supply reliable reference models for forensic work [2].

The domestic pig, (*Sus scrofa domesticus),* has been utilized extensively as an animal model in biomedical studies because of its physiological parallels to humans, such as its cardiovascular system [3]. Since the pig heart and the human heart are similar in both anatomy and physiology, the pig heart is a useful model to examine histological alterations in cardiac tissue [4]. We can obtain important insights into the postmortem modifications unique to cardiac tissue and develop parallels with human cardiac histology by examining the histological changes in pig hearts [1].

Understanding human physiological processes and postmortem changes in different tissues has been made possible by the use of animal models in forensic research. One such model that has gained popularity is Sus scrofa domesticus, also referred to as the domestic pig, because of its physiological parallels to humans and its accessibility as a research subject [1-4]. Pigs are a good model to study cardiac histological alterations because they share similarities with humans in terms of the circulatory system, including the anatomy and function of the heart.

Studies utilizing the pig model have demonstrated its effectiveness in evaluating histological changes in cardiac tissue. For example, a study by Smith, *et al.*, [5] examined the histological alterations in pig hearts after controlled ischemia, mimicking the conditions observed in myocardial infarction. The researchers found significant histological changes, including myocardial necrosis, cellular infiltration, and fibrosis, like those observed in human hearts following ischemic events.

Johnson *et al.*, [1] looked at the histological alterations in pig hearts that had been subjected to traumatic traumas, like blunt force trauma. The hemorrhage, myocardial fiber breakdown, and inflammatory response that the researchers saw in the cardiac tissue bore a striking resemblance to the alterations that occur in human hearts following severe cardiac injury.

The results demonstrate the value of using the pig model to investigate histological alterations in heart tissue for forensic investigations [1]. Gaining a thorough grasp of the postmortem histological alterations unique to pig hearts can help in correctly interpreting analogous alterations seen in human cardiac tissue during forensic examinations. This study intends to assess and document the histological alterations that occur in postmortem heart tissue using the *Sus scrofa domestica* model.

The heart as one of the visceral organs in the body can be seen in any crime scene involving death, and the estimation of time of death called the postmortem interval becomes imperative. This study was designed to fill such knowledge gap in Forensic Science, by exploring the decomposition science the heart inn successive postmortem stages (Fresh, Bloat, Active and advanced decay).

2.0 Materials and methods

2.1 Ethical Clearance

Ethical clearance was gotten from both University of Uyo and University of Cross River State Ethics and Research committees respectively prior to the commencement of this research.

2.2 Experimental Animals

This study cohort comprised of sixteen domestic pigs (*sus scrofa domestica*) gotten from a piggery at Okuku market. The range of the animals' weight ranges between 30-35Kg, and all the animals were sacrificed simultaneously.

2.3 Study design

This study cohort comprised of sixteen domestic pigs (*Sus Scrofa domesticus*) weighing between 30 to 35Kg, conducted at the department of Anatomy and Forensic Anthropology research facility (DAFARF) UNICROSS, Okuku Campus. This study assessed the histological postmortem alterations in cardiac tissue samples taken at regular intervals according to the study design, and from the sixteen porcines one sample each was collected.

The animals were euthanized using sharp axillary trauma and death was confirmed at about 1:14 pm and dissection cum evisceration of organ of interest followed immediately for all the five animals and tissue samples were collected from one animal model per day for the five days of the study duration. On day one (1), the first sample was taken at 1:58pm (Noon) which was our control, Sample 2 from the second model was taken at 2hours PMI at 3:58 pm (Noon), sample three was taken using 6hours PMI at 7:58pm (evening), sample 4 was taken using 10hours PMI at 11:58pm. On day two (2), the first sample was collected using 18hours PMI at 7:58am (morning), sample two was collected using 24hours PMI at 1:58pm (Noon), sample 3 was collected using 30hours PMI at 7:58pm (evening). At day three (3), the first sample was collected using 42hours PMI at 7:58am (morning), sample 2 was collected using 48hours PMI at 1:58pm (Noon), sample 3 was collected using 54hours PMI at 7:58pm (evening).

At day four (4), the first sample was collected using 66hours PMI at 7:58am, second sample was collected using 72hours PMI at 1:58pm (Noon), sample 3 was collected using 78hours PMI at 7:58pm (Noon).

At day three (5), the first sample was collected using 90hours PMI at 7:58am, sample2 was collected using 96hours PMI at 1:58pm (Noon), sample 3 was collected using 102 hours PMI at 7:58pm (evening). The samples are fixed in boun's fluid and later transferred to 70% ethanol after 6 hours. Dehydration of specimens was carries out using ascending grades of alcohol (70% ethanol for 15min, 90% for 15 min, 100% for 15min, 100% for 30min 100% for 45min). Samples are cleared using grades of xylene (1 xylene for 20min, 2 xylene for 20min, 3 xylene for 45min). infiltration was done using molten paraffin wax at 60 degrees

centigrade and allowed to cool for 20degres centigrade as follows (1 xylene for 30min, 2 xylene 30min, 3 xylene 45min). Tissue blocks were formed from embedding for easy microtomy. Sectioning of blocks is done using microtome machine to form smaller slices. Floating follows immediately to allow specimen spread since it must have formed ribbons. The specimen was mounted using DPX and routine staining techniques employed.

2.4 Histological tissue processing procedures

Histology is the microscopic study of animals and plant cell and tissue through staining and sectioning and examining them under a microscope (electron or light microscope). Histological studies are used in forensic investigations, autopsy and diagnosis and in education. It is extensively used in medicine especially in the study of diseased tissues to aid treatment [6]. The process of histological staining takes five key stages which involves; fixation, processing, embedding, sectioning and staining [7].

2.5 Histomorphometry assessment

The Experimental Animals were euthanized using sharp axillary trauma and the cardiac tissues from the sixteen animal models were eviscerated and preserved with bouin's fluid and transferred after 6 hours into 70% ethanol for histological preparation and examination. Myocytes cross sectioning areas of paraffin embedded tissue samples were stained with Hematoxylin and Eosin, Periodic acid Schiff and Masson's Trichrome stains [8].

2.6 Research analysis

After animal sacrifice, samples were collected using 0-102 hours PMI for five days (morning, afternoon and evening) and preserved in bouin's thereafter transferred to 70% ethanol after 6 hours. The samples were processed using routine tissue processing procedures and the following stains employed as seen below:

Hematoxylin and Eosin (H&E); this stains remains one of the principal and gold standard histological staining. It is a combination of two different stain; Hematoxylin and eosin. The Hematoxylin stains cell nuclei, giving it a purplish-blue coloration and eosin staining the extracellular matrix and cytoplasm giving it a pinkish coloration.

Periodic Acid Schiff (PAS); this is a special stain used during histological procedures to detect glycogen in tissues.

3.0 Results

3.1 Histology results

Examination of Haematoxylin and Eosin-stained cardiac sections showed gradual deterioration in the structural integrity starting from 10hours postmortem compared with sections obtained from 0-6hoiurs postmortem.

On the other hand, further examination of the H and E-stained cardiac sections showed severe disorientation in the structural integrity starting from 42hour postmortem, as compared with micrographs from 10hours postmortem. The figures 1A-3 and 2A-C respectively showed 24hourly photomicrographs of the cardiac tissues and

their level of progressive decay from 0hr to 102hrs respectively.

Similarly, the outcome of the histological tissues stained with Masson trichrome, showed a progressive depletion of cellular collagen fibers that is the connective tissue that holds the cardiac muscles together (figure 3A-C and 4A-C). Here also the decay rate appeared progressive and the level of collagen concentration decreased across the tissues as the postmortem interval increased.

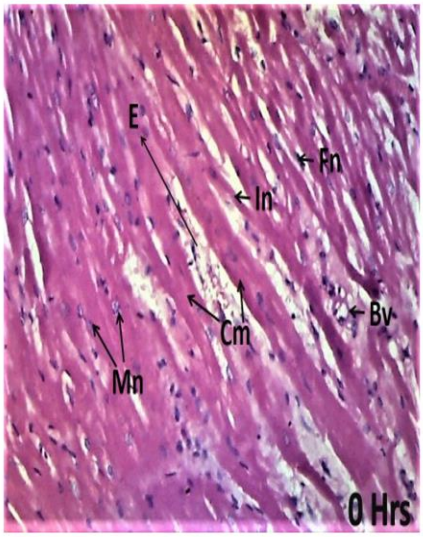

Figure 1A: Photomicrograph of a 0-Hour exposed heart tissue showing normal cardiac architecture with the myocardium having well-preserved cardiac myocytes (Cm), myocyte nuclei (Mn), fibrocyte nuclei (Fn), blood vessels (Bv), epimysium (E), and intercalated disc (In) (H&E x100).

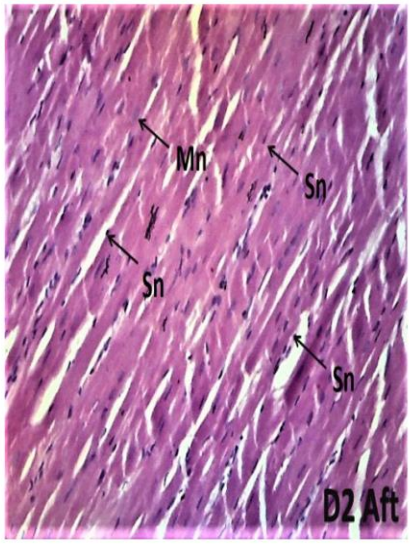

Figure 1B: Photomicrograph of the Day-2 afternoon exposed heart tissue showing disoriented cardiac architecture having myocyte nuclei (Mn), and shrinking nuclei (Sn) within the myocardium (H&E x100).

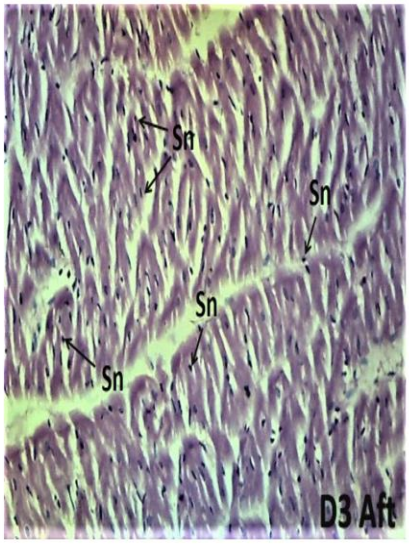

Figure 1C: Photomicrograph of the Day-3 afternoon exposed heart tissue showing disoriented cardiac architecture having shrinking nuclei (Sn) within the myocardium (H&E x100).

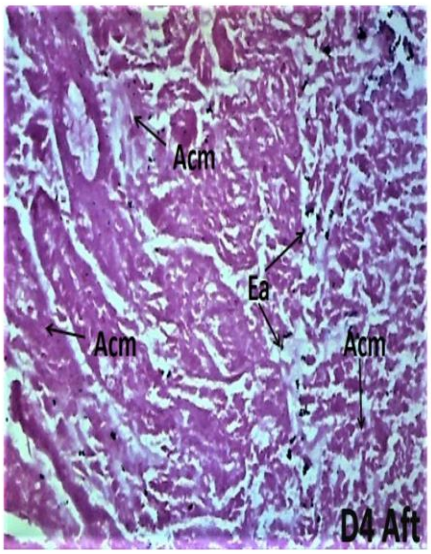

Figure 2A: Photomicrograph of the Day-4 afternoon exposed heart tissue showing disoriented cardiac architecture having wide presentation of shrinking nuclei (Sn) and anucleated cardiac myocytes (Acm), and exogenous artifact (Ea) within the myocardium (H&E x100).

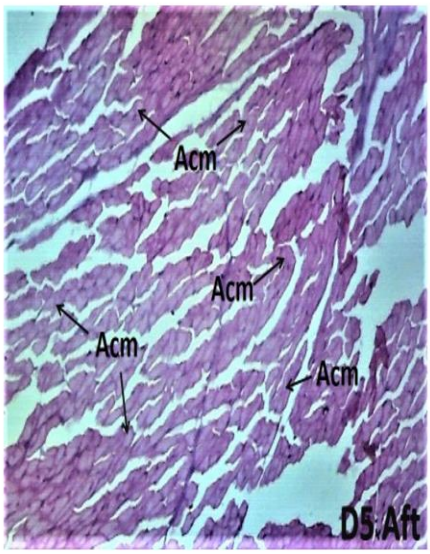

Figure 2B: Photomicrograph of the Day-5 afternoon exposed heart tissue showing disoriented cardiac architecture having wide presentation of anucleated cardiac myocytes (Acm) within the myocardium (H&E x100).

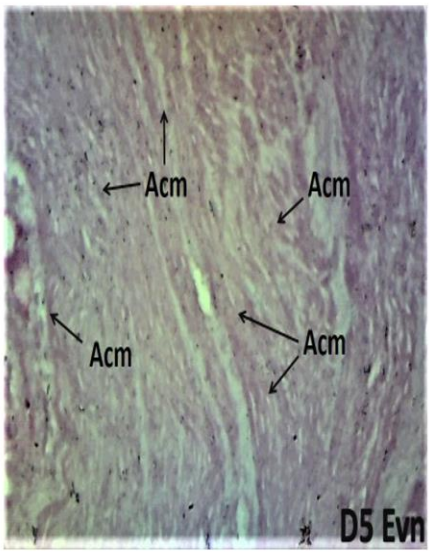

Figure 2C: Photomicrograph of of the Day-4 evening exposed heart tissue showing disoriented cardiac architecture having wide presentation of anucleated cardiac myocytes (Acm) within the myocardium (H&E x100).

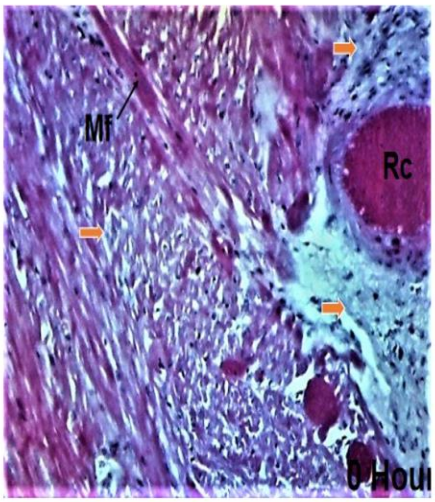

Figure 3A: Photomicrograph of a 0 Hour exposed heart presenting a normal connective tissue presence within the cardiac tissues with areas of collagen and fibrous tissue deposit (yellow arrow), muscle fibers (Mf) and red blood cell (Rc) (MT x100).

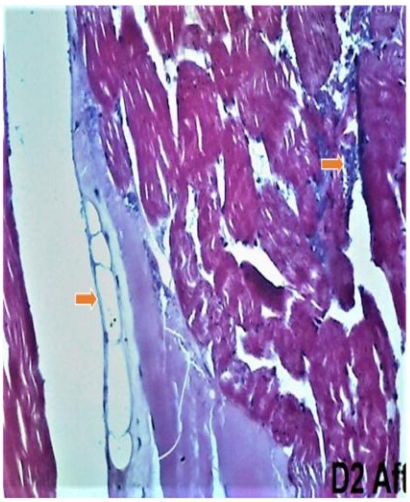

Figure 3B: Photomicrograph a day 2 afternoon exposed heart presenting a distorted connective tissue presence within the atrophying myocardium with decreasing areas of collagen and fibrous tissue deposit (yellow arrow) (MT x100).

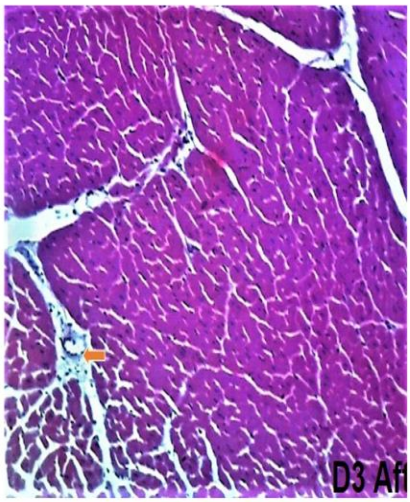

Figure 3C: Photomicrograph of a transverse section of a day 3 afternoon exposed heart presenting a distorted connective tissue presence within the atrophied myocardium with areas of decreased collagen and fibrous tissue deposit (yellow arrow) (MT x100)

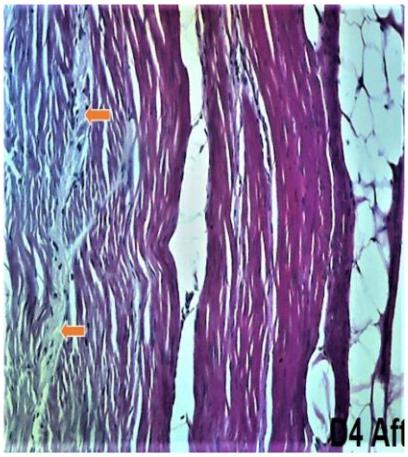

Figure 4A: Photomicrograph of a transverse section of a day 4 afternoon exposed heart presenting a distorted connective tissue presence within the atrophied myocardium with areas of decreased collagen and fibrous tissue deposit (yellow arrow) (MT x100).

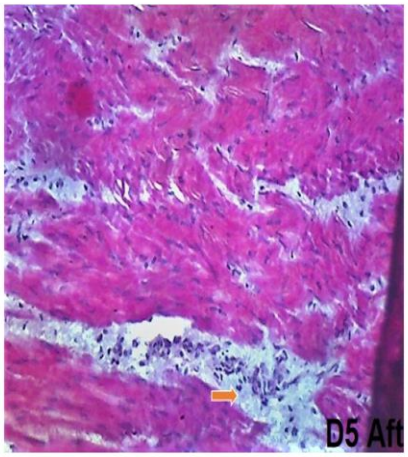

Figure 4B: Photomicrograph of day 5 afternoon exposed heart presenting a distorted connective tissue presence within the atrophied myocardium with areas of decreased collagen and fibrous tissue deposit (yellow arrow) (MT x100)

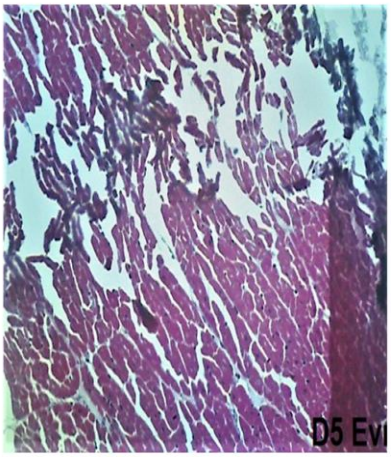

Figure 4C: Photomicrograph of day 5 evening exposed heart presenting no connective tissue within the atrophied myocardium with areas of no obvious collagen and fibrous tissue deposit (MT x100).

4.0 Discussion

This study attempted to evaluate the histological postmortem changes in cardiac tissues of *Sus Scrofa domesticus* forensic purposes and to estimate the time since death (PMI).

The normal course of a body's breakdown following death is known as postmortem alterations, and it starts at the cellular level[9]. The changes that started as soon as a person passes away continue for a long time and happen to different organs at varying rates [10]. Determining the postmortem interval (PMI), or time since death, requires an understanding of postmortem changes [10]. Determining the postmortem period is a crucial component of forensic medicine studies [11].

Estimating the postmortem interval (PMI) from the observable state of decomposition is a difficult problem for death investigators because the rate depends on a number of variables that are not well studied. For those who have passed away unexpectedly or as a result of natural causes, the date of death might not be as significant; but, for those whose lives have been wrongfully taken from

them, the PMI assessment can be crucial. To ease a positive identification using fingerprints, DNA, or dental records, a PMI can condense the lengthy list of missing persons [12].

After death, the deprivation of oxygen triggers a change of events which results in cell necrosis and the onset of autolysis and then putrefaction.

The present study provides valuable new knowledge pertaining to the establishment of PMI using successive histological changes of the heart exposed at various time intervals.

In the current investigation, a portion of the heart exposed to various postmortem periods showed a spectrum of histological alterations as seen below.

At day one (morning, afternoon and evening), using 0-10 hours PMI which conforms to fresh stages of decay, shows normal cardiac micro-architecture with myocytes nuclei, fibrocytes nuclei, blood vessels, cardiac myocytes, and epimyosium were all intact and mild effect observed at 10 hours PMI revealed shrinkage of nuclei and sparse areas of vacuolated myocytes.

At day two (morning, afternoon and evening), using 18-30 hours PMI which conforms to bloating stages of decay. The myocardium showed myocytes nuclei, shrinking nucleus and fibrocytes nuclei with severe effects.

At day three (morning, afternoon, and evening), using 42-54 hours PMI which conforms advance stages of decay. Notable observations within the myocardium were fibrocytes nuclei and wide presentation of shrinking nuclei which was a severe effect on the cardiac issue.

At day four (morning, afternoon and evening), using 66-78 hours PMI which connotes active stage of decay. Myocardium showed disoriented cardiac architecture with severe effect showing anucleated cardiac myocytes, shrinking nuclei.

A day five (morning, afternoon and evening), using 90-102 hours PMI which conforms to advance stages of decay. The myocardium presents severe effect with disoriented cardiac architecture showing wide presentations of anucleated cardiac myocytes.

However, the notable differential postmortem changes in cardiac tissue could be as a result of the effects of variable decomposition timeline which is in affiliation with the study of Zhiyuan *et al* [13]. A significant discovery here is the nuclei's shrinkage, in agreement with Zhiyuan *et al* [13] this implies that exposure to various PMI causes a notable histological alteration. But according to Zhiyuan *et al* [13], morphological alterations of the cardiac tissue structures after death reveal that the myocadiac cell structure was essentially complete between 0 and 45 hours after death and vanished at 156 hours PMI. This investigation confirmed the findings of Thent *et al* [14]. who reported aberrant cardiomyocytes nuclei, disorganized myofibers, and decreased deposits of connective tissues. Furthermore, the notable histological alterations in cardiac muscles, such as the vacuolization of myocytes and the shrinkage of nuclei, are also consistent with the conclusions made by Shah *et al* [15].

Additional noticeable moderate-to-severe alterations in the cardiac tissues, such as fibrosis, myocytes hypertrophy, and the absence of the cardiac myocytes nucleus (ACM) seen between day 3 to 5 (figures 2A-

C), were likewise consistent with the findings of Duncanson *et al* [16].

According to Becker *et al*. (2013), "understanding postmortem changes is necessary for determination of the postmortem interval (PMI) or time since death," the histological postmortem changes found from this study are critical for determining the time since death (TSD) of a deceased person.

5.0 Conclusion

The results of this study revealed evident histological changes in cardiac tissue across the postmortem timeline studied. In this study, the histological changes in the cardiac tissue have provided additional clues and information for forensic experts to build on and serve as a guide in establishment of postmortem interval. The changes described in this study can be relied upon in the prediction of PMI, which one of the vital parameters employed by any forensic investigator saddled with the responsibility of recovering individuals' identity and biological profiling in disaster victims' identification.

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