

PERICARDIUM DISORDERS IN SPRAGUE DAWLEY (SD) RATS INDUCED HIGH-FAT DIET (HFD)

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Abstract:

Background and objective: Pericardium disorders is rare and often asymptomatic but potentially lead to cardiac failure if left untreated. High-Fat Diet (HFD) is one of the contributors to the occurrence of cardiovascular diseases. This study focused on the observation of pathophysiology changes on pericardium of Sprague Dawley (SD) rats to assess the effects of dietary intakes in the initiation and development of the aforementioned disease. Methods: Ten Sprague Dawley (SD) rats were divided into HFD (n=5) as the treated group and Normal Diet (n=5) for the control samples over the course of 12 weeks. Daily tasks involved feeding and general cleaning in the animal house, whilst monthly duties were taking the body weight, body length and recording the Body Mass Index (BMI) for a monitoring and anthropometrical measurement. As for the microscopic study, the outer layer of heart tissues was selected for histological analysis using Hematoxylin and Eosin (H&E) staining. All photomicrograph processed using ImageJ software. Results: Histological analysis revealed significant abnormal cells growth which indicated inflammatory activities suggesting the possible cysts or tumors development on the pericardium of SD rats on HFD. Conclusion: Hypercaloric diet such as HFD played a crucial role in the event of pericardium disorders. The outcomes of this study will potentially provide more insight toward future research on the association between HFD and pericardial disorders. Hence, future advance study remains paramount.

Keywords —High-FatDiet(HFD),Inflammation,Pericardium,SpragueDawley(SD).

I. INTRODUCTION

Pericardium disorders often occurred chronically and asymptotically, although it is not considered as a life-threatening condition, it will eventually cause fatality if left untreated. The diagnosis rather complex since it's requires in-depth demographic settings and the need of an advanced medical technologies. The diseases exist in a form of pericarditis, myopericarditis, constrictive pericarditis, pericardial effusion, pericardial defects, and cardiac tamponade which creates huge challenges in term of management and potential

treatments [3]. Pericardium disease is associated with cardiac remodeling, it is, therefore, the excessive consumption of a High-Fat Diet (HFD) played a crucial role in metabolism and pathophysiology changes in the cardiovascular system causing various ranges of cardiac events including pericardium dysfunction [15]. Apart from that, socioeconomic status and lifestyles are among the well-known variables contributed to the risks of developing cardiac-related disorders. In addition, environmental factor significantly contributed to the occurrence of the aforementioned diseases and usually the evaluation and assessment of tissues

morphological performed via histomorphometry [15]. Among the current diagnostic option for cardiac-related diseases are imaging technology such as CT scan and MRI which commonly practiced and considered as a reliable source of diagnosis since it enabled physicians to perform full overview of cross-sectional structure of the pericardial layer. However, histopathological approach remains important as a supplementary method to diagnose the disease and it will provide all the essential pathology descriptions of pericardium dysfunction [15]. Therefore, this study focused on histology analysis of the Sprague Dawley rat (SD) rat heart tissues particularly on pericardium to identify any cells abnormalities and inflammations apart from pericardial infarction, constrictive pericarditis [1]. Our finding will potentially fulfil the criteria required for further advanced research in diagnostics and treatment of pericardial diseases due to HFD. Since most of the previous studies indicating the frequent utilization of imaging technology but limited in terms of histological approach [15].

II. METHODS

A. Ethical Approval

The research ethic was obtained from Management and Science University, Shah Alam, Malaysia (MSU-RMC-02/FR01/02/L3/020).

B. Study design

Ten random gender Sprague Dawley (SD) rat divided into two group used in this experiment, High-Fat Diet (HFD) and Normal Diet (ND). SD rats was maintained in a control environment which 22–24°C and 12 h light/dark cycles and housed polypropylene cages 2 rats per cage [5], [12]. HFD consists 60% fat mixture and 40% standard rat pellets whilst ND made up of pure rat pellet. Diet formulation was prepared based on diet dilutions method [4] from week 0, week 4, week 8 and week 12 prior to histological analysis.

TABLE I

BASELINE CHARACTERISTIC OF SD RATS (N=10) FOR 12 WEEKS

Week	High-Fat Diet (HFD)			Normal Diet (ND)		
	Body Weight (g)	Body Length (cm)	Body Mass Index (BMI)	Body Weight (g)	Body Length (cm)	Body Mass Index (BMI)
0	80.194 ±2.093	15.344 ±0.425	0.340 ±0.024	75.504 ±2.246	15.200 ±0.447	0.326 ±0.028
4	177.40 0 ±0.384	19.400 ±0.548	0.474 ±0.018	127.200 ±2.871	17.200 ±0.873	0.430 ±0.074
8	387.74 2 ±6.264	24.800 ±0.837	0.018 ±0.625	274.400 ±5.704	22.800 ±1.304	0.524 ±0.048
12	472.72 6 ±2.264	24.980 ±0.320	0.758 ±0.037	361.220 ±4.991	23.600 ±0.548	0.648 ±0.086

C. Tissues Histology

Histological analysis: As for the microscopic study, the outer layer of the heart tissues was isolated by cross-sectioning the organ; Fixation was done in 10% formaldehyde and left for 10 days incubation at room temperature. Dehydration involved samples immersion in a series of ethanol concentration and Xylene, embedded in paraffin wax and Sectioning in microtome with blade adjusted to 5µm in thickness and ribbon-like shape tissue sections were collected using glass microscopic slides and left to dry overnight at 40°C. Cardiac tissue staining was performed using Hematoxylin and Eosin (H&E) for the microscopic analysis [17].

D. Data analysis

All photomicrographic were processed using ImageJ [8] software.

III. RESULT AND DISCUSSION

E. Histology of Pericardium

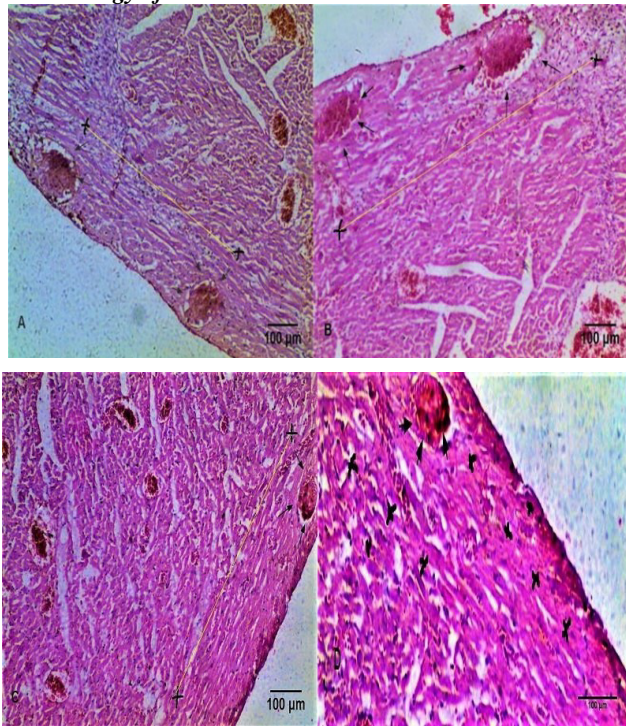


Figure 1 Photomicrographic A, B, C, and D show the outer layer (Pericardium) of SD rats induced with High-Fat Diet (HFD)

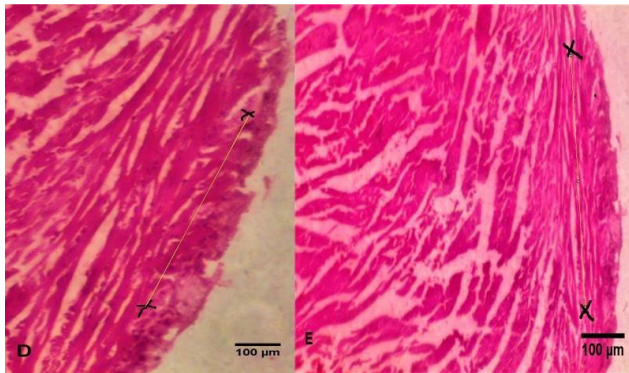


Figure 2 Microscopic observation for cardiac tissue (pericardium) of SD rats induced with Normal Diet (ND) using standard pellet

Histological analysis was performed using H&E staining on the outer layer of the selected heart tissues which focused on the pericardium. Based on the histology findings on the selected heart tissues

(fig 1), there was a very significant inflammatory activities on the cardiac tissues indicating the event such as thickening of the outer layer of heart organ and also potentially cardiac tissue injuries [16] & [18]. In addition, there were obvious signs of cells accumulation (pointed by arrows) as indicated in image A, B, C, D (see fig 1) which could be an initial sign of pericardium tumors [2] & [19]. Then, both images D and E (fig 2) severed as the control group and the outer layer of the heart tissues shown no significant abnormalities or inflammation.

This finding suggested that the high concentration of saturated fat has a great influence on the occurrence of pericardium disorders such as inflammation and abnormal cells growth on the outer layer of the SD rat's heart. The HFD composition was made up from 60% of the fat mixture formulation that was adapted based on the existing formula [6] and 40% from standard rat's pellet that was prepared using the diet dilutions method [4]. Then, the HFD was supplemented with cholesterol from pure duck yolk sources [7], [9], [10] & [11] to increase the level of fat in the food mixture. As a result, there was an obvious increase in BMI of SD rats from the start to the end of the experiment. The overall changes on SD rat's anthropometric measurement corresponded to the abnormal cells activities which led to the pericardium disorders in HFD group. On the other hand, the control group was fed with normal diet made up from standard gold pellet [5] has shown smaller changes in term of BMI in comparison to the treated group. In addition, since all rats were maintained under a controlled environment of 22–24°C with 12 hours light and dark cycles inside polypropylene cages in the animal house [5], it is, therefore assumed that the environmental factor definitely played a part in the occurrence of the cardiac disorders among the experimental rats.

In this study, pericardium analysis performed specifically using standard H&E staining to obtain the most possible accurate visualization in order to examine the existence of the aforementioned disorders. As for the treated group, the staining shows blue and pinkish colors representing nuclei

and cytoplasm indicating cells were concentrated and form a mass accumulation within the pericardium layer (fig 1). Previous studies reported that an unbalance cell growth is one of the early signs of cysts or tumors development, and they are normally categorized into primary (benign and/or malignant) as well as metastatic type [19]. Apart from that, previous study suggested that the pericardial cysts are more common compare to diverticula; cyst comes in a fixed size and approximately the same density as water whilst diverticula appear in varies sizes and shapes depending on where they located. The common cause of pericardial cyst is due to infections of mycobacterium tuberculosis, rheumatic fever, and post-cardiac surgery trauma. They can potentially grow progressively and remain asymptomatic, although some patients might experience shortness of breath and chest pain during the process. Among the available treatment options are including percutaneous aspiration and thoracoscopic surgery [1], [19].

Overall, the histology of the pericardium shown mild to moderate signs of cell abnormalities within the group of rats induced with HFD, suggesting the potential development of cysts or tumors cells due to the hypercaloric dietary intakes.

IV. CONCLUSION

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This study summarized the occurrence of chronic inflammation and potential cysts or tumors development specifically around the pericardium was largely due to the excessive hypercaloric food consumption. In addition, this finding could potentially fulfil the criteria required for further advanced research in the prognostic and treatment of pericardium diseases. However, the specific calcification of the suspected cells was not concluded and therefore future studies is required to provide an in-depth understanding and details of these abnormalities along with a wider range of

parameters remains paramount to provide a solid justification to the outcomes of this study.

CONFLICT OF INTEREST

No conflict of interest.

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APPENDIX

Equation 1

Body mass index (BMI) $[\text{kgm}]^{-2} = (\text{Body weight (kg)} / (\text{Body length (m)}^2))$

Equation 2

Lee index $(\sqrt[3]{\text{kg/m}}) = (\sqrt[3]{(\text{Body weight (kg)} / (\text{Body Length (m)})^2))$

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