



Mortality of Sprague Dawley (SD) Rat on Long Term High-Fat Diet (HFD)

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Abstract

Background and Objective: Heart failure is linked with metabolic syndrome due to an unbalanced dietary intake. Previous studies suggest that cardiac dysfunction is related to chronic inflammation due to injury within the heart muscle. This study focused on the examination and sudden death of Sprague Dawley (SD) rat which was on long-term High-Fat Diet (HFD). The outcomes will potentially provide an insight into future research in sudden death due to HFD. Methods: Total of 15 SD rats grouped into ten HFD and five on normal diet (ND); Female SD rats on HFD underwent physical examination, tissue analysis 12 hours post-death using Hematoxylin & Eosin (H&E) followed by morphological assessment and SD rats from ND used as control. Microscopic images processed using ImageJ and data analysis performed in SPSS software. Results: No significant physical trauma prior to death, however on the 60th day SD rat suffered chronic inflammation to cardiac tissues with Lee Index (LI) of 0.30, indicated the occurrence of Myocardial infarction (MI). The average mean between the size area of inflammation and the region on cardiac tissue is (834.19 ± 103.41) and 94.2% of inflammation activities explained by the four random regions of cardiac tissue ($R^2 = 0.942$, $F(1, 2) = 32.401$, $p < 0.05$), with every one unit increase in the size area of inflammation, the infected region of cardiac tissue is estimated to increase at 0.970 ($\beta = 0.970$, $p < 0.05$). Conclusion: Chronic inflammation resulted in myocardial injuries led to mortality of SD rats largely due to HFD.

Keywords: Sprague Dawley; Inflammation; Histology; Myocardial Infraction.

Introduction

It has been scientifically proven that socioeconomic status, lifestyle, and environmental factors are among the known variables contributing to the susceptibility of developing cardiovascular diseases (Pokhrel & Guotian, 2017).

Metabolic syndrome corresponds to the type of diet we are on which is often considered the main cause of heart-related disorders (Kershaw & Flier., 2004). Previous reports suggested that a large proportion of global

deaths are due to cardiac events, including stroke and myocardial infarction (MI) affecting all levels of the world's population (Gurung *et al.*, 2018). Cardiovascular remodeling is an inflammatory disease associated with the excessive uptake of Low-Density Lipoprotein (LDL) mainly due to a High-Fat Diet (HFD) triggering the recruitment of immune cells leading toward the formation of atherosclerotic plaques and affecting the pathophysiology of the heart muscle causing cardiac failure (Thent *et al.*, 2012). Historically obesity was

first introduced in rats with the utilization of ad libitum as in the semi-liquid form of diet and, according to the nutritional obesity HFD formulation of which fat composition at 50% was successfully induced obesity in rats (Sorsiky, 1999). In addition, HFD consist of more than 10% of fat composition which is believed to be hypercaloric as it is composed of both saturated fatty acid and monounsaturated fatty acids causing obesity; this condition leads to the breakdown of triglyceride and is absorbed into the small intestine and transported via the circulatory system, thus affecting the health and vital function of the heart (Hariri & Thibault, 2010).

Therefore, this study was undertaken in order to identify the influence of diet toward the sudden death of the female Sprague Dawley (SD) rat which was on long-term HFD. The finding of this study will potentially fulfill the criteria required for further advanced research into the relationship between dietary and cardiovascular remodeling.

Materials and Methods

Materials

Measuring tape, digital weighing scale, syringe, surgical knife, surgical scissors, microtome machine, microtome blade, histological cassette, Paraplast wax, microscopic slide, coverslip, optic microscope, 10% Formaldehyde, Hematoxylin, Eosin Y (1% alcoholic solution), Potassium acetate, Hydrochloric acid, Ethanol, Xylene.

Methods

Ethical consideration:

The ethical committee approval was obtained from the research committee of Management

& Science University (MSU-RMC-02/FR01/02/L3/020).

Study object:

Fifteen (15) female Sprague Dawley (SD) rat was taken for the study. Ten (10) of the SD rats grouped as HFD (positive control) and five (5) on Normal Diet (negative control).

Specimen examination:

SD rat was dissected for examination approximately 12 hours post- death, according to the standard dissection protocol (Fenton & Dowling., 1953).

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 sample 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 Haematoxylin and Eosin (H&E) to visualize inflammatory activities. Four different regions of the outer layer of cardiac muscle were subjected to microscopic observation according to (Sowash, 2009) & (Slaoui & Fiette, 2011).

Data analysis:

Photomicrographs were taken and processed using ImageJ software (Ferreira & Rasband., 2012). Statistical analysis and interpretation performed using SPSS software (Lorimer, 2019).

Table 1. Baseline Characteristic of Experimental SD rats according to groups.

Parameter	Treated (HFD)			Control (ND)		
	Treatment Day0 (Mean±SD)	Treatment Day30 (Mean±SD)	Treatment Day60 (Mean±SD)	Control Day0 (Mean±SD)	Control Day30 (Mean±SD)	Control Day60 (Mean±SD)
Body Weight (kg)	0.077±0.003	0.186±0.008	0.381±0.006	0.072±0.001	0.173±0.003	0.256±0.013
Body Length (m)	0.158±0.005	0.185±0.010	0.260±0.014	0.151±0.004	0.190±0.007	0.228±0.011
Body Mass Index (kg/m ²)	0.308±0.234	0.551±0.076	0.570±0.065	0.315±0.016	0.484±0.047	0.495±0.023

Results

Specimen examination

Figure 1 shows a series of Images, 1 to 4 were taken as the pre-examination of female SD rats. The initial observation revealed no signs of physical trauma on the victim. Although a good amount of fat deposition was found around the heart, this indicated the classic physical changes due to HFD. The study object was a part of a group of SD rats fed with a diet containing high levels of fat mixture with caloric manipulation for the duration of 90 days. However, this rat was unable to survive and reported dead on the 60th day. The cause of sudden death was unclear but suspected largely due to MI.

The baseline characteristic as shown in table 2 suggesting abnormalities in terms of rat's vital signs in just 8 weeks. It is known that the dietary intake whereby HFD made from 60%

of fat mixture, 40% from standard rat's pellet which was prepared according to the dilution method (Lousinha et al., 2015).

In addition, lipid profiling was performed for monitoring purposes throughout the duration of the diet program; according to the data presented in table 3, LDL level is way below the High-Density Lipoprotein (HDL) and Triglyceride (TG) level on day 30, and the LI was within the normal limit. This indicated that the victim's negative feedback of the HFD started to take place afterward. LI of more than 0.30 indicated the individual most probably suffered from various cardiac-related diseases and stands a higher risk of heart failure (Delorme et al., 1981). In this study, the LI level of SD rats on day 60th was 0.30. This suggested that the loss of life was most likely due to heart failure because of the chronic inflammation resulting in tremendous pressure within the cardiac tissue.

Figure 1. Photograph of SD rat taken before and after dissection approximately 12 hours post-death.



Table 2. Baseline characteristic of SD rats on day 60.

Parameters	Values	Standardized Values
Body Weight Day 60	386.16 g	0.39 kg
Body Length Day 60	25.50 cm	0.26 m
BMI Day 60	0.64	0.64
Lee Index Day 60 (LI)	0.30	0.30

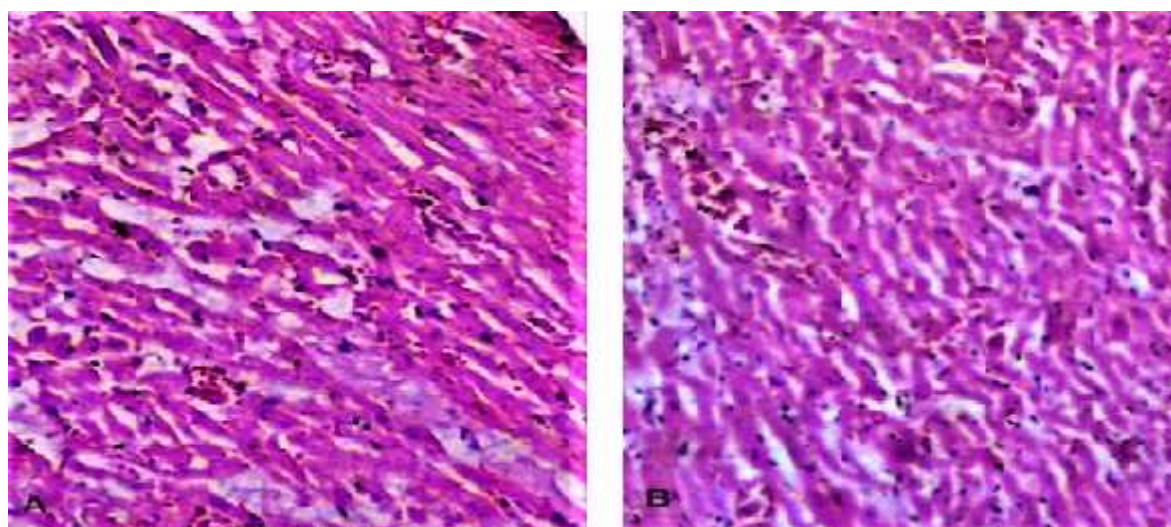
Table 3. The last record (day 30) of lipid level (mg/dL) and LI prior to death.

LDL	HDL	TG	LI
19.8	58	111	0.29

Histology of Cardiac Tissues

Histological analysis was performed using H&E staining on the outer layer of the SD rat cardiac tissues in order to validate the pre-examination of the SD rat (see figure 2 & figure 3). Based on the histology findings on the selected heart tissues (figure 2), there were significant inflammatory activities in the cardiac tissues, this indicated the occurrence of myocardial injuries as one of the most likely causes of death (Thent *et al.*, 2012). Previous reports strongly agreed that excessive amounts of fat and calorific content in food consumption potentially lead to various heart diseases such as chronic inflammation of the cardiac muscles causing MI (Thent *et al.*, 2012). Similarly concerning the mortality of the SD rat which suspected that there was an insufficient amount of blood passing through the heart due to myocardial injuries as the

result of the inflammation. Previous studies highlighted there is a strong correlation between the anthropometrical changes and mechanism of the central nervous system which focused on the hypothalamus, neuropeptides, and hormones (Kiess *et al.*, 2008) & (Rodriguez- Correa *et al.*, 2020). The inflammation found in the cardiac tissues indicated the involvement of inflammatory signaling molecules including Interleukin-6 and Tumour Necrosis Factor Alpha (TNF α) synthesized from endocrine organ suggested that chronic inflammatory disease is due to obesity (Kiess *et al.*, 2008) & (Rodriguez-Correa *et al.*, 2020); (Skelton *et al.*, 2006) & (Konopelnyuk *et al.*, 2015). Thus, the SD rat in this study has developed obesity on week 8 and suffered from chronic inflammation to the cardiac muscle leading to MI (Kiess *et al.*, 2008).

Figure 2. All photomicrograph region A, B, C, D shows a high level of inflammation on the cardiac muscle (SD Rat) induced with HFD.

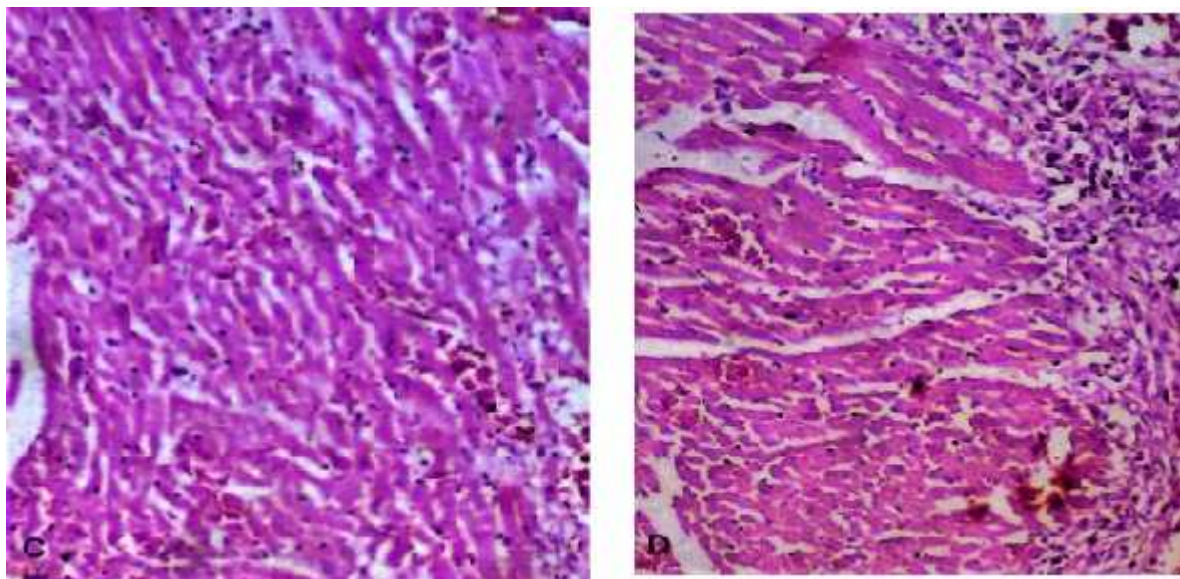
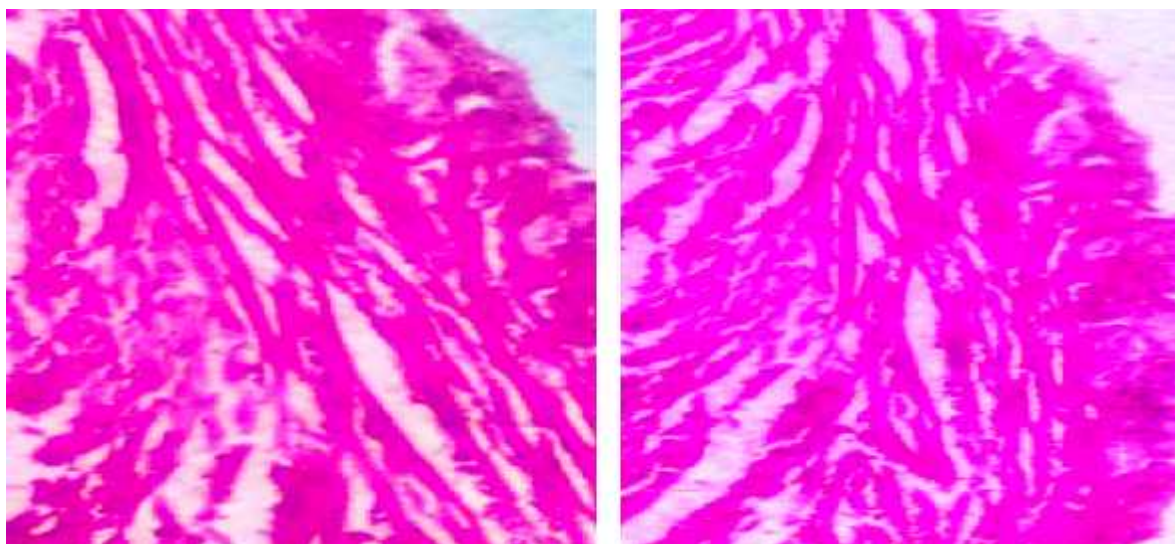


Figure 3. Photomicrograph of SD rats on Normal Diet (Control group).



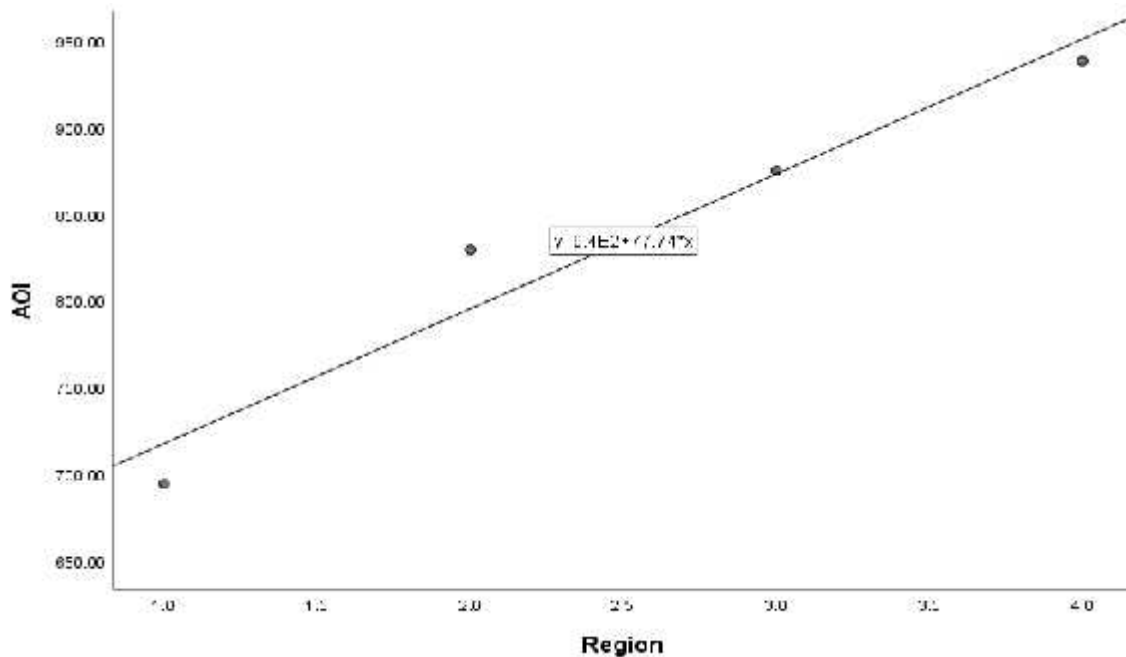
Tissues Morphological Assessment

Four random areas of cardiac tissue namely region A made up of $694.26 \mu\text{m}^2$, region B region is $829.36 \mu\text{m}^2$, region C is $874.97 \mu\text{m}^2$, while region D is $938.19 \mu\text{m}^2$ (see figure 4). The average mean between the size area of inflammation and the random region of cardiac tissue is (834.19 ± 103.41) and *Pearson's* test shown a strong positive correlation between the size area of inflammation and the random region on cardiac tissue which was statistically significant ($r= 0.978$, $p= 0.015$). Statistically, ANOVA test revealed that 94.2% of inflammation activities explained by the four random region of cardiac tissue [$R^2= 0.942$, $F(1, 2)= 32.401$, $p< 0.05$]. According to the results of the standardized Beta weight, for

each unit increase in the size area of inflammation, the region of cardiac tissue is estimated to increase at 0.970 ($=0.970$, $p< 0.05$), whereby the 95% confidence interval values range between 18.978 to 136.502.

The tissue morphology evaluation shows a strong relationship between the size area of inflammation and the location within the cardiac muscle since the tissue selections were conducted randomly and then used in histological analysis. However, it is necessary for any further related studies to select and target specific regions of the cardiac muscle in order to evaluate the probability of inflammation occurring within a different part of heart muscles.

Figure 4. Scatter plot above representing the relationship between the Size Area of Inflammation (AOI) and the random region of cardiac tissue of SD rat.



Conclusion

The main objective of this study was to determine the cause of sudden death in a female SD rat on long-term HFD. It can be concluded that the dietary composition significantly contributed toward the cardiac tissue inflammation and HFD played a crucial role in the overall well-being of the rat. The conclusion made in this study was based on several parameters including specimen examination, anthropometrical evaluation, tissue analysis, and morphological findings. All outcomes highly supported the role of hypercaloric diet contributed toward cardiac muscle inflammation which in turn caused cardiac injury and subsequently lead to mortality. However, further studies remain paramount by utilizing different variables together with a wider range of parameters to come up with solid justification on the effects of HFD in cardiac inflammation that led to the MI occurrence.

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Conflicts of Interest:

The authors declare no conflict of interest.

Appendix

Equation 1.

Body mass index (BMI), $\left[\frac{\text{kg}}{\text{cm}^2} \right]^{-2} = \frac{\text{Body weight (kg)}}{\text{Body length (m)}^2}$.

Equation 2.

Lee index $\left(\frac{\text{kg}}{\text{m}} \right) = \left(\frac{\text{Body weight (kg)}}{\text{Body Length (m)}} \right)^3$.

Equation 3.

LDL cholesterol = TC - (HDL cholesterol) - (TG/5).

Abbreviations

Lee Index (LI), Myocardial Infarction (MI), Sprague Dawley (SD), Triglyceride (TG).

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