HISTOPATHOLOGICAL AND HISTOCHEMICAL ANALYSIS OF HEART MUSCLE AND AORTA OF WISTAR RATS EXPOSED TO HIGH-FAT DIET

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Abstract:

Introduction: Obesity is a crucial component of metabolic syndrome, which also includes dysglycemia, high blood pressure, elevated triglyceride levels, and low levels of HDL cholesterol. A metabolic syndrome is a group of interconnected risk factors for diabetes and cardiovascular disease. Hyperlipidemia caused by a high-fat diet is one of the most prominent risk factors for atherosclerosis and coronary heart disease around the world. For more than 20 years, statins have been the most routinely prescribed drugs for the treatment of hyperlipidemia

Aim: The aim of the study is to elucidate the Histopathological and histochemical analysis of heart muscle and aorta of Wistar rats exposed to a high-fat diet

Materials and method: A total of 4 formalin fixed paraffin embedded tissue blocks of Wistar rat samples that were exposed to high-fat diet and 2 control rat samples. Four lysine-coated slides were obtained from each block and used for further processing.

Results: The expression of Alpha SMA is significant in High-fat diet samples compared to control samples. The expression of S100 is increased compared to control samples. Increased fibrosis was seen.

Conclusion: Overall, the findings obtained herein suggest exposure to HFD can alter cardiac integrity and induce heart fibrosis.

Keywords: Wistar rats, heart, aorta, histopathology, Histochemical, high fat diet.

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1. Introduction:

Obesity is a crucial component of metabolic syndrome, which also includes dysglycemia, high blood pressure, elevated triglyceride levels, and low levels of HDL cholesterol. A metabolic syndrome is a group of interconnected risk factors for diabetes and cardiovascular disease.(1)

Over the last few decades, obesity and its related metabolic side effects have become an epidemic. Several experimental animal models have been created to gain a deeper knowledge of these illnesses and assess prospective metabolic syndrome therapies (2).
Although obesity has a multifaceted etiology, the fact that it is becoming more common shows that environmental and behavioral factors, rather than genetic changes, have been the main causes of the obesity epidemic. Polygenetic animal models of diet-induced obesity have thus been utilized more frequently than monogenetic models(3).

In addition to being a sickness, obesity is also a major contributor to a number of fatal illnesses, such as insulin resistance, oxidative stress and inflammation, hypertension, and cardiovascular mortality. Obesity is a "medical disease in which extra body fat accumulates to the point where it may have a detrimental influence on health," according to the World Health Organization. When regular calorie intake and regular energy expenditure are out of balance over time, a complex disease like this one develops. The adult prevalence of overweight and obesity has dramatically increased recently, according to a Chinese study in the areas of nutrition and health. Another analysis indicates that compared to the frequency recorded in 1986, the prevalence of obesity worldwide has increased by roughly a factor of two since 2014. Being overweight rather than underweight has contributed to an increase in mortality and morbidity. The metabolic syndrome, which includes high blood pressure, central obesity, atherogenic dyslipidemia, oxidative stress, and a proinflammatory and prothrombotic condition, is generally brought on by a high-fat (HF) diet(4).

The new millennium has seen the birth of a modern epidemic, coronary heart disease, which is one of the most common types of cardiovascular illness and has devastating effects for human health globally. Heart disease and stroke, according to a World Health Organization (WHO) assessment, will become the major causes of mortality and disability worldwide. In the year 2023, India is expected to be the world's "Death Capital," with the biggest number of people suffering from CVD. Increased consumption of added sugars, particularly fructose, has been the focus of much study as a probable contribution to the current epidemic over the previous few decades, as has changed in food habits and food composition(5).

Obesity is a worldwide condition caused by a combination of genetic, environmental, and behavioral factors, and it is linked to high rates of morbidity and mortality. It's characterized as having more body fat than lean body mass. Obesity is clinically defined as a high BMI (body mass index) (BMI). Individuals with a BMI of 25 to 29.9 kg/m2 are considered overweight, while those with a BMI of 30 kg/m2 or more are considered obese. Obesity has been linked to a variety of health issues, including high blood pressure, heart disease, diabetes, stroke, osteoarthritis, sleep apnea, premature death, and a lower quality of life. A nutritious diet aids in the maintenance or improvement of general health by providing the body with vital nutrients such as hydration, macronutrients, micronutrients, and sufficient calories(6). Our team has extensive knowledge and research experience that has translate into high quality publications (7–16))

Hyperlipidemia caused by a high-fat diet is one of the most prominent risk factors for atherosclerosis and coronary heart disease around the world. For more than 20 years, statins have been the most routinely prescribed drugs for the treatment of hyperlipidemia(17).

Over the past few decades, cardiovascular diseases (CVD) have sharply increased around the world. According to some predictions, the yearly CVD mortality rate may increase to 22.2 million people by 2030. The extensive use of pesticides and other environmental pollutants could be contributing to the ongoing rise in CVD. Recent studies have demonstrated that consuming food and water that have been contaminated with these agricultural pollutants, as well as direct occupational exposure to pesticides, all contribute to the development of CVD. According to a number of indicators, lipid profile issues, inflammatory conditions, and oxidative stress brought on by pesticide exposure may all have a role in cardiovascular events. The precise mechanics, however, have not yet been completely clarified(17,18).

Diabetes mellitus refers to a collection of disorders that impact the way in which the body utilizes blood sugar (glucose). Glucose is an essential energy source for the cells that comprise muscles and tissues. It is also the primary source of fuel for

the brain. The primary aetiology of each form of diabetes varies (19). The aim of the study is to elucidate the Histopathological and histochemical analysis of heart muscle and aorta of Wistar rats exposed to high-fat diet

2. Material and method:

A total of 4 formalin fixed paraffin embedded tissue blocks of wistar rat samples that were exposed to high fat diet and 2 control rat samples . Four lysine-coated slides were obtained from each block and used for further processing.

Preparation of buffers:
Tris buffer was prepared by dissolving 8 grams of sodium chloride and 0.6 grams of Tris buffer in 1000 ml of distilled water and the pH was adjusted to 7 by adding sodium chloride. Citrate buffer was prepared by dissolving 1.05 grams of citric acid monohydrate in 500 ml of distilled water and the pH was adjusted to 6.5 by adding diluted hydrochloric acid. The pH of these solutions was adjusted using the pH meter.

Normal Protocol:
Sections of 2.5µmin thickness were cut and mounted on a lysine-coated slide after removing from the incubator the slides were kept for deparaffinization by placing in two changes of xylene, each change lasting for ten minutes followed by isopropyl alcohol for 5 minutes. The antigen retrieval was done as follows, the slides were placed in a citrate buffer and kept in a pressure cooker for two whistles. The slides were then cooled and agitated in Tris buffer for two changes of five minutes each, after which the slides were marked with a PAP pen which creates a water-repellent barrier that keeps staining reagents localized on the tissue sections. The slides were placed in the humidor after applying the peroxide block for 30 minutes. The slides were again washed in two changes of Tris buffer. After which the slides were covered with the primary antibody, Alpha- SMA, CK and s- 100 for sixty minutes after which the slides were washed with Tris buffer. The secondary antibody was applied for thirty minutes and again washed with Tris buffer. The slides were then covered with a DAB buffer (Novolink) in the ratio of 1:20 for a minute. The slides were then washed in Tris buffer and the counterstain was achieved with haematoxylin, the slides were dried and mounted.

3. Results:

In our study, we compared the Histopathological and histochemical analysis of heart muscle and aorta of Wistar rats exposed to high-fat diets. When compared to the controlled group the value of expression of Alpha SMA is significantly higher in High-fat diet samples compared to control samples and the expression of S100 is increased compared to control samples. In a high-fat diet, increased fibrosis was seen.

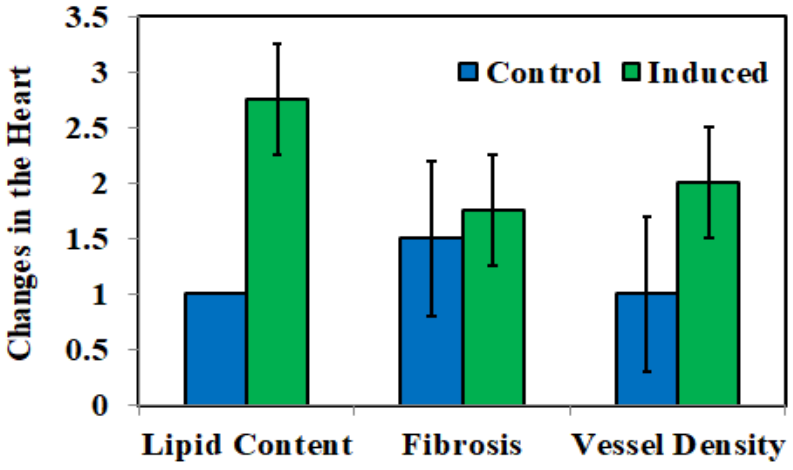

Figure 1: Heart and aorta alterations in wistar rats after 72 hours. The Y-axis depicts the mean values, while the X-axis represents the cellular changes in the heart. The colour blue represents the control group, whereas the colour green represents the induced or test samples.

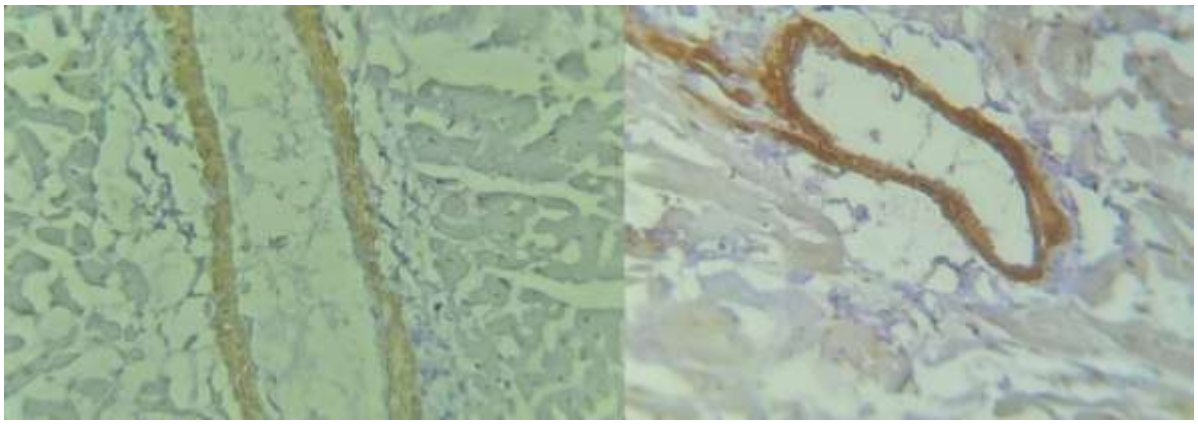

Figure 1: Shows the IHC staining of alpha- SMA of the test rat samples.

	Mean		**Standard deviation**	
	Control	**Induced**	**Control**	**Induced**
Lipids	1	1.75	0	0.5
fibrosis	1.5	1.75	0.7	0.5
density	1	1.75	0	0.5

Table 1: The table depicts the mean and standard deviation of the various degenerative alterations observed in the heart and aorta of the samples and the control rats.

4. Discussion:

For decades, rodent models of obesity, dyslipidemia, and insulin resistance have been employed. It has been shown that high-fat eating disorders mirror metabolic syndrome in humans, and this may also apply to cardiovascular repercussions. Essentially, all laboratory rodent species are susceptible to acquire metabolic abnormalities under such feeding conditions. The sensitivity to a high-fat diet, or the severity of the metabolic sickness caused by a particular diet, depends more on the mouse strain and the food than on the species. In contrast to Wistar rats and mice, A/J mice do not develop fat and insulin resistance. As far as we are aware, the specific mechanism behind the high-fat tolerance exhibited by several rat and mouse strains remains unknown.(20).

The majority of studies on the "pure" metabolic effects of high-fat diets in wild-type animals have been conducted on rats, while a large number of studies on mice have examined genetic modifications in response to a high-fat diet. Nonetheless, the diversity of individual outcomes reflects the variability of the related experimental designs. In these latter research, the severity of the metabolic disturbance (weight gain, glucose, insulin, and adipokine levels, insulin resistance, etc.) described by previous authors corresponds well with our findings (weight gain, glucose, insulin, and adipokine levels, insulin resistance, etc.).(21,22).

Given the paucity of previous, complete, high-fat diet comparisons, the primary objective of this study was to examine the impact of high-fat meals differing primarily by their main fat component on the development of metabolic syndrome in Wistar rats. Based on the animal phenotype and the characteristics of glucose metabolism and insulin action, it appears that both the HF-L and HF-O diets induced the most pronounced obesity and insulin resistance symptoms. Compared to animals given coconut fat, fish oil, or normal rats, these animals gained more weight, had higher plasma glucose levels, and insulin-induced glucose clearance was less effective. (22).

Following the development of obesity, an increase in glucose level can be detected. According to a previous study, persistent exposure to high doses of fatty acids causes an increase in fatty acid oxidation and a decrease in glucose oxidation in animals consuming high fat diets(23). The

consumption of high saturated fat was also found to increase the expression of genes involved in hepatic lipid synthesis and impair glucose metabolism as evidenced by decreased glucose transporter-4 activity, suppressed glucose transporter-4 expression, inhibited expression of hepatic glycolytic and lipogenic enzymes, and impaired insulin signaling(24).

Increases in TG, cholesterol, and LDL were brought on by consuming a high-fat diet, but HDL levels dropped. Additionally, it is evident that obese-induced rats had greater levels of LDL and TG compared to the negative control group. By moving fat from the liver to adipose tissue, LDL accelerates the breakdown of triacylglycerol. 60% to 70% of the serum's overall cholesterol is carried by it. Increased liver VLDL-triglyceride production into circulation may be the source of the hypertriglyceridemia condition brought on by a high-fat diet. Unsaturated triglycerides were said to upregulate LDL receptors more than saturated triglycerides did. As a result, it was proposed that dietary fatty acids may cause alterations in the lipids of cellular membranes(25).

5. Conclusion:

Overall, the findings obtained herein suggest exposure to HFD can alter cardiac integrity and induce heart fibrosis. Of note, current findings It is highly relevant to understanding the effects of lifetime exposure to food contaminants and obese diets, Especially given the global situation of high fat consumption. In our study we found that Histopathological and histochemical of heart muscle and aorta of Wistar rats exposed to a high fat diet showed the expression of SMA was significantly high and the expression of S100 increased.

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