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Histopathological And Immunohistochemical Analysis Of Testicular Tissues Of Wistar Rats Exposed To A High-Fat Diet

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ABSTRACT

INTRODUCTION: Histopathology is the study of the signs of the disease using the microscopic examination of a biopsy or surgical specimen that is processed and fixed onto glass slides. Histochemistry is an important technique that is used for the visualization of biological structures. A high-fat diet (HFD) is a diet consisting of at least 35% of total calories consumed from fats, both unsaturated and saturated. AIM: To elucidate Histopathological and immunohistochemical analysis of testicular tissues of wistar rats exposed to high fat diet. MATERIALS AND METHODS: Healthy adult male albino Wistar rats were maintained under standard laboratory conditions with ad libitum access to food and water, in accordance with Institutional Animal Ethics Committee guidelines (IAEC no: BRULAC/SDCH/SIMATS/IAEC/02-2019/015). The animals were divided into two experimental groups: Group 1 (n = 2): Subjected to death by submersion in water. Group 2 (n = 4): Clinically sacrificed as controls. Following a 72-hour post-mortem interval, skin and epithelial tissues from both groups were collected and analyzed. RESULTS: There is an Increased lipid deposition when compared to control group. There is narrowing of seminiferous tubules. Significant Reduction in cellular proliferation. Cell shrinkage is also seen. CONCLUSION: We have identified several candidate proteins and conducted pathway analysis around the effects of the HF diet on the testis providing novel insights which are not previously described....

Keywords: Wistar rats, high fat diet, seminiferous diet, cell proliferation

INTRODUCTION

Histopathology is the study of disease symptoms through microscopic examination of a biopsy or surgical specimen that has been processed and fixed onto glass slides. The sections are stained with one or more stains in order to visualize different components of the tissue under a microscope. IHC, or ImmunoHistoChemistry, is a special staining technique used on fresh or frozen breast cancer tissue obtained during a biopsy. IHC determines whether cancer cells have receptors and hormone receptors on their surface. This information is crucial in treatment planning

Obesity, insulin resistance, hypertension, and dyslipidemia are all symptoms of the "metabolic syndrome." (1)This condition affects 20-40% of the population in developed countries, and its prevalence is expected to rise further in the coming decades. Nutrition and physical activity are both important factors in its manifestation, but the exact chain of causation is unknown.(2) In 1959, the first description of a "high-fat diet" to induce obesity through nutritional intervention was published. Subsequent research has shown that high-fat diets promote hyperglycemia and whole-body insulin resistance, and many researchers have investigated their effects on muscle and liver physiology as well as insulin signal transduction(3). Based on this experience, it is widely accepted that high-fat diets can be used to create a reliable rodent model of metabolic syndrome, complete with insulin resistance and impaired -cell function(4).

The testicle was surrounded by a capsule or tunica albuginea with a mesothelial cell lining on the outside. Thin fibrovascular septae descended from the tunica albuginea and continued as inter-tubular connective tissue septae in the testis parenchyma(5). There was no histological difference between the right and left side testes. In humans, adolescent dietary behavior appears to contribute to obesity, including low meal frequency, and skipping breakfast, and a high consumption of sugar-sweetened beverages. Obesity's effect on pubertal timing has been extensively studied in the literature. However, no

studies have been conducted to investigate the effect of adolescent obesity on the morphology of adult testis and sperm(6). Data from our group show that a high fat diet during adolescence promotes obesity and subsequent deregulation of glucose metabolism.(6,7)

However, it is unknown whether this has any impact on reproduction system structure or function(8). Several studies have found that physical exercise helps with metabolism and obesity control(9). Exercise has long been thought to be an effective stimulant for controlling body weight gain and metabolic dysfunction in organisms predisposed to obesity(10). Exercise performed during adulthood has also been shown to improve sperm quality and fertility potential in rat offspring of obese dams(11). Hypothesized that exposure to a high fat diet during adolescence causes long-term dysfunction of the metabolic and reproductive systems, but that physical exercise improves function. In this context, the present study aimed to investigate the effect of a high fat diet on testicular tissues of male wistar rats.

MATERIALS AND METHODS:

Animals were maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethics Committee (IAEC no: BRULAC/SDCH/SIMATS/IAEC/02-2019/015). Healthy male albino rats of Wistar strain (Rattus norvegicus) weighing 180–210 g (150–180 days old) were used in this study. Animals were obtained and maintained in clean polypropylene cages under specific humidity (65±5%) and temperature (27±2 °C) with a constant 12 h light and 12 h dark schedule at the Central animal house facility, Saveetha Dental College and Hospitals, Chennai-77. They were fed with a standard rat pellet diet (Lipton India, Mumbai, India), and clean drinking water was made available ad libitum.

Experimental design

Healthy adult male albino rats were divided into two groups, where one group contains two wistar rats and the other group contains four wistar rats. Group 1: The rats have been submerged in water for death. Group 2: The rats are clinically sacrificed on a regular basis. In a time period of 72 hours the rats were sent to post-mortem analysis. During analysis the skin and epithelial tissues were studied.

RESULTS:

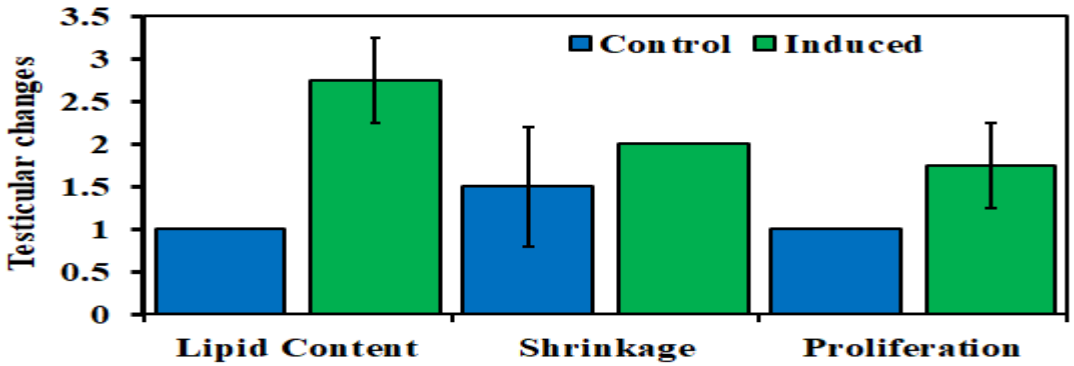
Histopathological the following results were seen such as ,there is an increased lipid deposition in seminiferous tubules which causes the narrowing of seminiferous tubules, there is a significant reduction in cellular proliferation and cell shrinkage is also observed. From graph 1, we can conclude that lipid content in seminiferous tubules of induced rats were significantly higher than the control group of rats. The cellular shrinkage is also significantly higher in induced rats than control rats. In rats induced with a high fat diet, there is an increased cellular proliferation than the controlled group.

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Lipid	Control	2	1.0000	.00000	.00000
	Induced	4	2.7500	.50000	.25000
Shrinkage	Control	2	1.5000	.70711	.50000
	Induced	4	2.0000	.00000	.00000
Proliferartion	Control	2	1.0000	.00000	.00000
	Induced	4	1.7500	.50000	.25000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Lipid	Equal variances assumed	4.000	.116	-4.667	4	.010	-1.75000	.37500	-2.79117	-.70883
	Equal variances not assumed			-7.000	3.000	.006	-1.75000	.25000	-2.54561	-.95439
Shrinkage	Equal variances assumed	.	.	-1.633	4	.178	-.50000	.30619	-1.35011	.35011
	Equal variances not assumed			-1.000	1.000	.500	-.50000	.50000	-6.85310	5.85310
Proliferartion	Equal variances assumed	4.000	.116	-2.000	4	.116	-.75000	.37500	-1.79117	.29117
	Equal variances not assumed			-3.000	3.000	.058	-.75000	.25000	-1.54561	.04561



Graph 1: This bar graph shows testicular changes with lipid content, cell shrinkage and cellular proliferation.

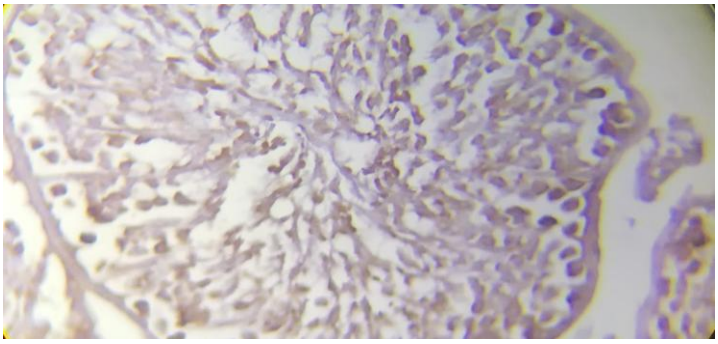


FIGURE 1

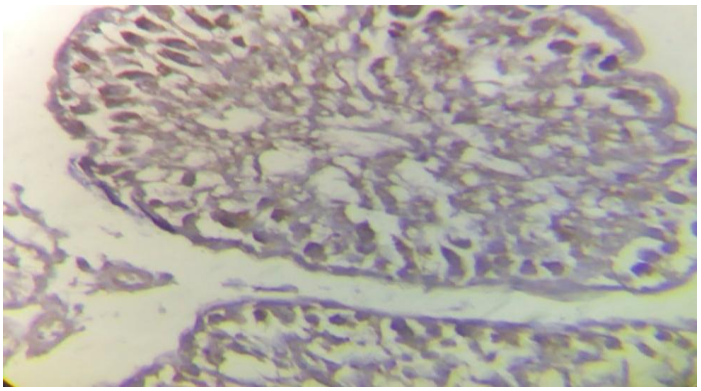


FIGURE 2

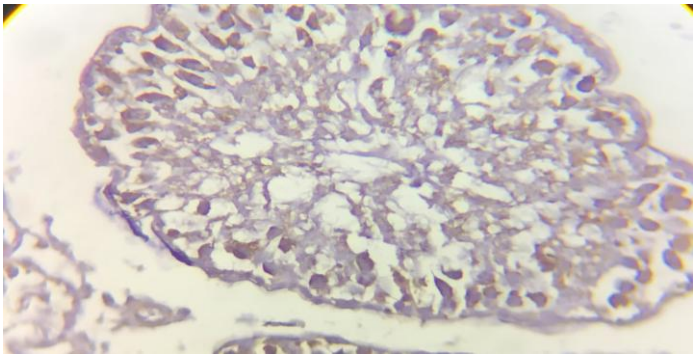


FIGURE 3

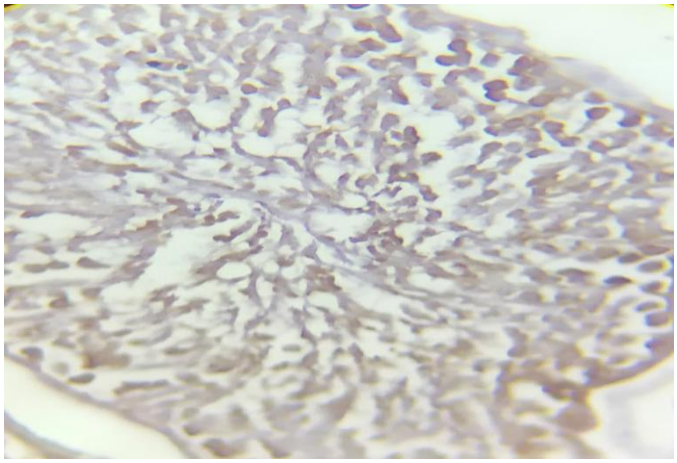


FIGURE 4

DISCUSSION:

From the above results it is clear that narrowing of seminiferous tubules is seen. Some previous studies stated that in comparison to controls, HFD-fed rats had significantly higher 8-OHdG content in sperm and testis, higher testicular SI, lower testicular weight, and higher SOD and GPx activity(12). Surprisingly, HFD supplementation appeared to reduce 8-OHdG in sperm and testis (22% and 24.3%, respectively), testicular SI and MDA content (28% and 40%, respectively),(13) testicular weight (24%), SOD and GPX activity (30% and 70%, respectively), and GSH content (19%)(14). Furthermore, regardless of diet, supplementation had a significant impact on increasing testicular folate content(15).

There is a significant reduction of cellular proliferation in high fat diet induced rats than controlled rats. All HF groups had an increase in genital fat deposits, confirming obesity. Serum glucose, insulin, and total cholesterol levels were higher in the HF-S and HF-SP groups(16). Thus, different concentrations of saturated fat (50% and 25% of total energy in the diet) were capable of causing metabolic changes(17). Recent evidence in humans has linked metabolic syndrome to hypogonadism(18). Testicular tissues and spermatozoa are extremely vulnerable to reactive oxygen species and lipid peroxidation. According to some studies, oxidative stress causes sperm membrane lipid peroxidation, which impairs sperm motility and sperm-oocyte interaction(5).

In high fat diet induced rats there is an increase in cellular shrinkage when compared to controlled rats. Histological examination revealed significant distortion of the seminiferous tubules in chronic HF diet-fed mice, as well as a decrease in germ cell numbers, particularly spermatogonial stem cells and maturing spermatids, with effects on the meiotic index(19). Sertoli cells, which are essential for germ cell survival, were reduced in number. As a result, the HF diet affects not only germ cell development but also Sertoli cells, and the two are likely to be linked(19). A review of the proteomic data revealed information about the biological pathways affected by the HF diet in the testis. Because proteins do not function in isolation, a protein-protein interaction analysis revealed high interaction scores, implying participation in a network of proteins with overlapping functions.

CONCLUSION:

Previous studies focused mainly on the abnormalities that occurred at the protein level and as a result, we have identified several candidate proteins and conducted pathway analysis around the effects of HF diet on the testis providing novel insights which are not previously described..

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