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Histological Study on The Effects of Orlistat on Left Ventricular Myocardium of High Fat Diet-Fed Adult Male Wistar Rats

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ABSTRACT

Obesity-induced by a high-fat diet in animal studies recapitulates the human form of obesity. Obesity is a serious public health concern, a risk factor for many diseases, including cardiovascular disorders. Our study evaluated the impact of orlistat on the left ventricular myocardium in high fat diet-induced obese adult Wistar rats. Our study included fifteen adult male Wistar rats weighing between (130-150g). The rats following environmental acclimatization were assigned into three groups A-C (n=5 each), the control (standard rat diet), high-fat diet only, high-fat diet, and orlistat as A, B, and C, respectively. The rats were placed on diets for eight weeks, which performed the sacrifice and histological procedures. The heart and left ventricles were excised and fixed in 10% buffered formalin solution for histological studies. Body Mass Index (BMI) of the high-fat diet-only group markedly increased ($P<0.05$) relative to the control and the orlistat-treated groups. Heart weight and relative heart weights were not significantly different across the groups. Histological examination of the left ventricular myocardium with the hematoxylin and eosin stain revealed significant deviation from the regular pattern in the high-fat diet-only group. The orlistat-treated group showed a near-normal left ventricular myocardial histoarchitectural pattern. Orlistat ameliorated left ventricular myocardial alteration, restored elastic tissue component, and reversed increased collagen deposition in high fat diet-induced obese male Wistar rats.

Key words: Histomorphology, Orlistat, High-fat diet, Myocardium, Heart.

INTRODUCTION

In 2015, an estimated number of about 17.7 million people died from cardiovascular disorders (CVDs) and this population represents about 31% of all deaths from CVDs globally (Zhu et al. 2019). Several factors which include and are not limited to sedentary lifestyles, diets are believed to influence the development of heart-related diseases (Zhu et al. 2019).

In the last 40 years, the burden of obesity has significantly increased in both developing and developed countries of the world (WHO 2020). Obesity as defined by the World Health Organization (WHO) is the acquisition of excess fat that negatively impacts one's health (WHO 2020). Also, obesity is known to originate from an imbalance between caloric intake and energy expenditure promoting an expansion of the adipose tissue. There is an established association between excess body fat and impairment in mitochondrial function with its attendant consequences on malonyl-CoA production, which invariably causes a reduction in GLUT4 efficiency (Coelho et al. 2011). Worthy of emphasis is the fact that obesity increases the risk for the development of cardiovascular diseases; heart failure and coronary heart diseases (CHDs). One of the reported mechanisms by which obesity increases puts one at risk for cardiovascular disease development involves, changes in body composition which could negatively affect hemodynamic stability leading to structural and functional heart changes (Coelho et al. 2011). This in turn could alter the myocardial structural integrity leading to left ventricular malfunction. The high-fat diet-induced obesity model has over-time been the model of choice for studying obesity in animal models.

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Asides from the negative impact of a high-fat diet on the structural integrity of the heart, there is also an association between the intake of diets rich in high fat and hyperphagia. The dietary fat type seems to play a germane role because saturated fats positively correlate to a positive fat balance and likewise omental adipose tissue accumulation as compared to other fats (Coelho et al. 2011). Polyunsaturated fats, omega-3 and omega-6 likely increases the rate of energy expenditure and lower energy intake by various mechanisms such as hormone-sensitive lipase, activation of peroxisome proliferator-activated receptor α (PPARα), (Coelho et al. 2011). Orlistat is an anti-obesity medication or a weight-reducing agent, whose mechanism of action is by inhibiting gastric and pancreatic lipases in the gastrointestinal tract which in turn leads to a decrease in systemic fat absorption. The major adverse effects of orlistat have been on the gastrointestinal tract which eventually gets resolved following continuous usage (Jean-Pierre et al. 2015). This study, one of its kind will help to establish or provide information on the histomorphological impact of orlistat on the left ventricular myocardium in high fat diet-induced obese adult male Wistar rats.

MATERIALS AND METHODS

Ethical consideration for this study was obtained from the local institutional committee. All experimental procedures for this study were per the procedures laid down by the institutional animal care and use committee (IACUC).

Orlistat, 120mg was procured from a local pharmaceutical store (Aromokeye & Co Ltd Pharmaceuticals/veterinary livestock drugs & Agrochemical Ilorin, Kwara State). Healthy male Wistar rats (*Rattus norvegicus*) of a total of fifteen, weighing between 130 to 150g were procured from the Animal Holdings of the Department of Anatomy, Afe Babalola University, Ado-Ekiti and were utilized for this study. The rats were housed in the Animal Holdings of Afe Babalola University, Ado-Ekiti. The rats were habituated for a period of two weeks before the commencement to the study. Experimental rats were randomized into 3 groups A-C (n=5 each). The rats were allowed to acclimatize for two weeks under the natural 12hrs, dark, and light periods. Rats in group A received standard laboratory rat pellet while rats in groups B and C were placed on a high-fat diet and high-fat diet + orlistat respectively for eight weeks. Rats in all groups had free access to water. The experimental diets were formulated using the modified method of Zuhar (2005), at the animal food manufacturing company (ACE factory in Osogbo) in Osun state.

Determination of body weights was weekly with the aid of the weighing scale (Metler Toledo, P163)

Percentage weight change was calculated using:

$$\frac{W_2 - W_1}{W_1} \times 100\%$$

Where W1= initial weight and W2 is the weight at sacrifice (Final weight)

The Relative organ weights were determined by the formula:

$$\frac{\text{Organ weight}}{\text{Final body weight}}$$

Body Mass Index was determined by the Lee's Index:

$$\frac{weight\ of\ the\ rat}{height\ (naso-anal\ length)\ of\ the\ rat} = \text{g/cm}^2$$

Hematoxylin and Eosin and Verhoeff-van-Gieson stains were used for assessment of the myocardial morphology, collagen and elastic fibres in the excised left ventricular tissues. The stained sections were viewed under a binocular microscope (Olympus) interfaced with a 5.1-megapixel MV550 camera. Digital photomicrographs were taken and archived.

GraphPad Prism 5 (Version 6.1; Graphpad Software Inc., San Diego, CA) was utilized for data analysis. Data analysis was done by one-way ANOVA followed by *posthoc* for mean comparisons using the Student Newman-Keuls test. Statistically significant difference was set at $P<0.05$.

RESULTS

Bodyweight significantly increased ($F=7.450$; $P=0.0079$) in experimental groups when compared to the control. *Post hoc* analysis showed that the bodyweight of the high fat diet-fed group was significantly higher compared to the standard rat diet and orlistat-treated groups (Fig. 1).

The body mass index (BMI) of the high-fat diet only group was significantly higher ($F=7.450$; $P=0.0079$) when compared to the control and group C (Table 2). The relative heart weight was not significantly different ($F=0.2500$; $P=0.7865$) among the groups relative to the control.

The normal histology of the cardiac fibers could be seen in the light micrograph sections of the control rats as indicated in Fig. 2A. The control group displayed a consistent and regular arrangement of myocardial fibers. However, in the high-fat diet-only group (Fig. 2B), there was deviation from the normal regular arrangement and morphological pattern of myofibrils. As evident is evidence of tissue necrosis and disoriented nuclei. In the histological presentations of the orlistat treated group as seen in Fig. 2C. This group showed a gradual restoration of the normal myocardial histological pattern. As compared to the control group, there was reduction in elastic fibers distribution in

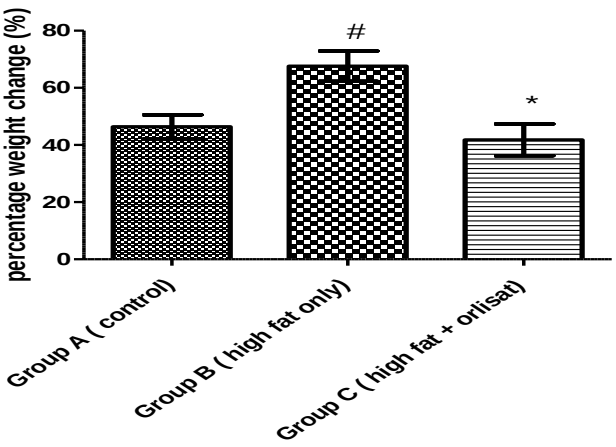

Fig. 1: Effect of high-fat diet and orlistat on the body weight. n=3, values are expressed in % weight change±SEM. # = relative to control at $P<0.05$; *= relative to group B (high fat diet) at $P<0.05$.

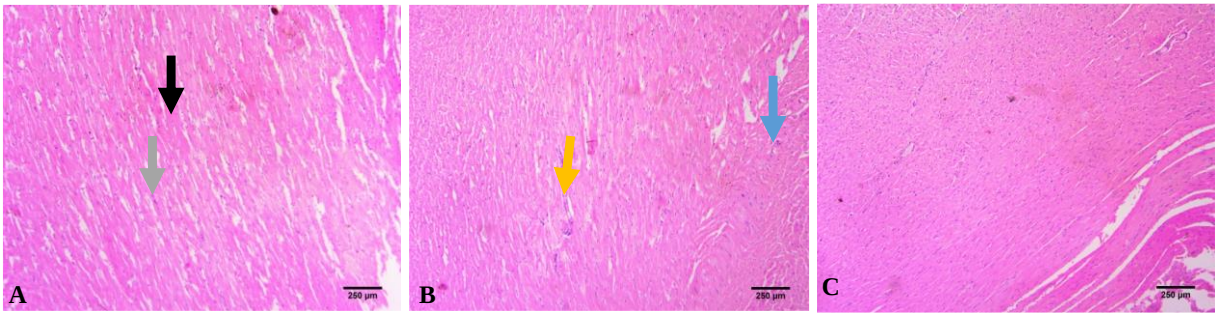

Fig. 2: Representative photomicrographs of the longitudinal section of the left ventricle of rats showing arrangements of cardiac muscles of group A (control). Nucleus of the myocytes is represented (green arrow) and muscle fiber is also indicated (black arrow) in group B (high-fat diet only), inflammatory cells (purple arrow), and areas of necrotic changes is identified (blue arrow). Hematoxylin and Eosin stain (x 200).

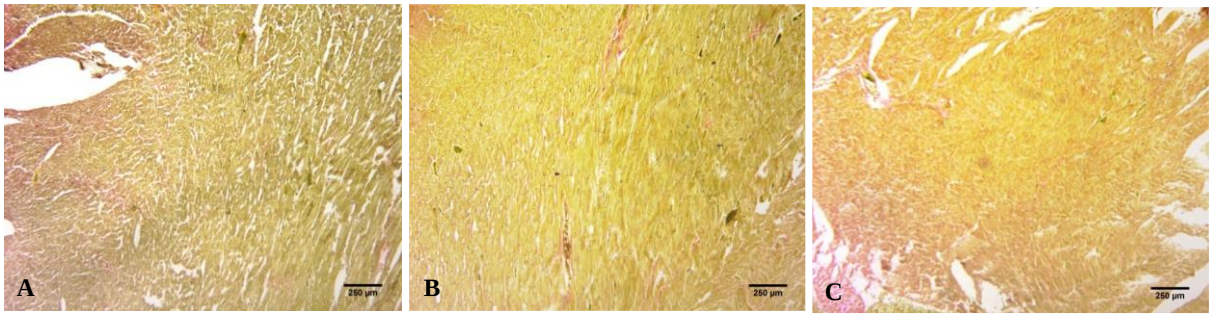

Fig. 3: Representative photomicrographs of the longitudinal sections of the left ventricle of rats showing distribution of abundant elastic fibres and sparse distribution of collagen fibres of group A (control), group B rats and group C. Note the sparse elastic fiber deposit (black) and abundant collagen fiber (red) in group B. Verhoeff-van-gieson stain (x 200).

Table 1: Grouping and experimental design

Groups	Agents	Duration
A	Standard rat diet	8 weeks
B	High-fat diet only	8 weeks
C	High-fat diet + orlistat (100mg/kg) dose	8 weeks

Table 2: The Effect of High Fat Diet and Orlistat on Relative Heart Weight

Groups	Heart's Absolute Weight (g)	Relative Heart Weight (%) ±SEM	Body Mass Index (BMI) (g/cm^2)	
			Baseline	Final
A (Control)	0.66±0.03	0.30±0.00	0.50±0.02	0.54±0.02
B (High fat fed only)	0.71±0.07	0.33±0.03	0.49±0.02	0.80±0.04$^{\#*}$
C (High Fat Fed + Orlistat)	0.63±0.09	0.30±0.06	0.54±0.04	0.58±0.03

Values are expressed as relative heart weight (%) ± SEM, no significant difference across the groups ($P<0.05$). n=3.

the high-fat diet-only group (Fig. 3B). The orlistat-treated group (Fig. 3C), displayed a significant improvement in the arrangement and distribution of the elastic fibers. Areas with increased collagen fibers distribution were noted in the histological sections of the left ventricular myocardium in the high-fat diet-only group (Fig. 3B).

DISCUSSION

High-fat diet-induced obesity in animal studies recapitulates obesity as seen in humans. Obesity poses a serious public health concern, being a risk factor for so many diseases including cardiovascular disorders. Our study aimed to evaluate the impact of orlistat on the left ventricular myocardium in high fat diet-induced obese adult Wistar rats. This could modify the metabolic disturbances produced by high caloric intake in those rats and the result showed that HFD induced a significant increase in the BMI and body weight gain across the groups relative to the control. This was as a result of the high caloric intake in the form of the high-fat diet. These results were in the same direction with Matos et al. (2005), Rezq and El-Khamisy (2011) with the report of Muls et al. (2001). Muls et al. (2001) and Mamikutty et al. (2014) stated that the normal BMI for male Wistar rats was in the range of 0.45±0.02g/cm^2 to 0.68±0.05g/cm^2. This present study showed that the high-fat diet only had a higher BMI of 0.80g/cm^2 when compared with control and high-fat diet + orlistat of 0.54g/cm^2 and 0.58g/cm^2 respectively. It has been reported in experimental studies that orlistat is highly efficient when given in combination with a high-fat diet. It is a reversible lipase inhibitor that acts by inhibiting the absorption of dietary fats (Muls et al. (2001) and this is a major mechanism by which it reduces weight. Therefore, the orlistat treatment would have resulted in a reduction in body weight, adipose tissues, and serum lipids as compared with the HFD group (Muls et al. (2001). Unlike the body weight, there was no significant increase or decrease in the relative heart weight of the rats across groups.

Verhoeff - van Gieson's stained sections showed that a high-fat diet significantly depleted elastic fibers deposition in the high-fat diet alone group relative to control. The normal functioning of the vascular system is a function of the elastic fibers. Their depletion has been associated with the development of cardiovascular diseases (Hiroshi 2011). Komolafe et al. (2009) had earlier reported that when that the sparse distribution of elastic fibers likely reduces the tensile strength and elasticity of the vascular structures which could be a basis for the development of cardiovascular issues. Whereas orlistat attenuated the damage caused by a high-fat diet by restoring the elastic fiber deposition as seen in the Verhoeff - van Gieson's micrographs of the groups D and E. Cardiac tissues are the high collagen regions that have formed to replace the dead cells caused by tissue necrosis as seen in (Fig. 3B and 4B). The maintenance of the extracellular matrix and the secretion of ground substances of the active connective tissues cells is a function of fibroblasts. Cardiac fibroblasts synthesize extracellular collagen type I and III in the left ventricle myocardium. Cardiac fibrosis is established to be involved in the pathophysiology of hypertension and cardiac hypertrophy (Ichihara et al. 2006) resulting in the impairment of left ventricle relaxation (Cingolani et al. 2003). Orlistat prevented myocardial histoarchitectural derangement in group C by restoring the antioxidant properties of the cells, as it had also been earlier reported, that orlistat is highly efficient when given in combination with a high-fat diet (Mahmoud and Elnour 2013).

Conclusion

Our study, one of its kind established that the anti-obesity medication (orlistat) ameliorated left ventricular myocardial alteration, restored elastic tissue component, and reversed increased collagen deposition which could possesses the propensity to alter left ventricular myocardial function in high fat diet-induced obese male Wistar rats.

Author's Contribution

ELA initiated the research and supervised various stages of the work. SOS was involved in the design of the experiment and supervised various stages of the work. EME was involved in the bulk of the research work and writing of the article. KOA was involved in the design of the experiment and proof-read the article. IMS was involve in tissue processing. OOR was responsible for the special fed for high fat diet. AAZ was involved in the writing and editing of article.

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