Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 12 (2)

Histological Studies of Effects of Orlistat on the Liver of High Fat Diet Adult Male Wistar Rats

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ABSTRACT

Fats consist of a wide group of compounds, which soluble in organic solvents and insoluble in water. This study is designed to evaluate the impact of high fat diet on the liver of the adult wistar rats. A total number of fifteen wistar rats with an average weight of 130-150g were purchased from the Animal House Handling Unit of Afe Babalola University Ado Ekiti, and divided into 3 groups. Group A was fed with Normal pellet, Group B with High fat Diet only, Group C with high fat diet + Ecoslim (orlistat) for 8 weeks. At the end of the experiment animals were sacrificed and liver of each animal was obtained and set in a buffered 10% formalin solution for histological studies. One-way ANOVA revealed that, there was significant difference in weight change and body mass index (BMI) across all experimental groups ($F = 7.450$; $p = 0.0079$). Post hoc analysis showed that weight of high fat fed group was significantly lower than the vehicle treated group. The weight reduction induced by high fat fed was significantly and reversed by orlistart administration ($p<0.05$). Histological findings revealed alterations in high fat diet alone group which was reversed by orlistat administration in group C.

The rats that received High fat diet during the experiment had a higher liver and body weight, with greater nucleus of hepatocytes, as well as an increased visceral fat. High fat diet also caused histometric changes in liver.

Keywords: *Histological; Orlistat; High fat diet; Liver*

INTRODUCTION

Human body contains certain amounts of fats in the ratio which depends significantly on the gender and age, and consists of essential fats involved in quite a number of physiological functions, as well as stored fats, mainly triacylglycerols, responsible for energy supply when necessary[1].

An excess of body fat tissue may be related not only to energy intake and energy expenditure in humans, but also to the type of diet, especially high-fat diets (HFD), which may lead to various metabolic alterations such as hyperphagia in humans, reduced lipolytic activity in fat tissue, reduction in leptin secretion and/or sensitivity, hypothalamic neuron apoptosis, impairment of mitochondrial metabolism, insulin resistance, and obesity[2]. The balance of each nutrient seems to involve a rigorous control to adjust its intake to its oxidation. An increase in carbohydrate and/or protein consumption is accompanied by increased oxidation rates of both nutrients. On the other hand, the balance between fat consumption and oxidation rates is not so tightly regulated and depends on the type of fatty acids[2].

It has been reported that using orlistat will produce significant weight loss and has been confirmed by long-term studies[3]. Orlistat is a gastrointestinal tract lipase inhibitor which decreases intestinal fat absorption by up to 30%[4]. Based on these clinical and safety characteristics in adult populations, it was believed that orlistat may be a useful adjunct to diet, exercise, and behavioral counseling in the treatment of obese adolescents[4]. Obesity itself is also associated with non-alcoholic fatty liver disease, and evidence from case series suggests that orlistat might improve liver function in such patients. No evidence of a higher rate of severe liver impairment events in those patients using orlistat, and their study assured that, though abnormal liver function is common in patients who are obese, it is not likely to be caused by orlistat[3]. This study will help to generate relevant information on the histomorphological impact of High fat diet on the liver of adult male wistar rats.

MATERIALS AND METHOD

Animal Care and Management: Fifteen male wistar rats were purchased from the Animal house handling unit of Afe Babalola university and divided into 3

groups, the animals were allowed to acclimatize for a period of one week under normal jurisdictions of acclimatization and have access to rodent chow, water and exposed to 12hrs by dark and light period.

A total of 15 adult male Wistar rats were used for this study. The average weight of the animals was 130-150g. They were distributed randomly into 3 groups (A-C). Group A animals were fed with normal pellet only, group B animals were fed with High fat diet and Group C animals were fed with High fat diet and Ecoslim for 8 weeks.

S/N	GROUPS	AGENTS	DURATION
1	A	Normal pellet	8 WEEKS
2	B	High fat Diet only	8 WEEKS
3	C	High fat Diet + Orlistat (100mg/kg) dose	8 WEEKS

Preparation of High Fat Diet: High fat diet was processed in the animal food manufacturing company (ACE factory in Osogbo) in Osun state, using the Formula: maize bran 1.08, groundnut cake 4, vit c 0.1, limestone 0.12, soya full fat 18, dicalcium phosphate 0.6, salt 0.1, and maize white 1[5].

Drug: Highly active anti-obesity drug (Ecoslim/Orlistat/Xenical 120mg) was gotten from the Aromokeye&co.ltd Pharmaceuticals/veterinary livestock drugs & Agrochemical. Ilorin, Kwara State.

Determination of Body Weight and Sacrifice of Animals: Body weights were taking at the beginning, 3rd week and last day of the experiment. The Animals were sacrificed on the last day of the experiment by cervical dislocation, blood samples were collected through the cardiac puncture and the collected blood samples were centrifuged, the plasma was then decanted from the blood into another sample bottle for biochemical analysis. The Liver was harvested and weighed using the gallenhamp electronic balance (MP 10001) weighing balance and fixed in 10% formal-saline for histological studies.

Percentage weight change was calculated using:

$$\frac{\text{Final weight} - \text{Initial weight} \times 100}{\text{Initial weight}}$$

Body Mass Index:

$$\frac{\text{weight}}{\text{height of the Animal in square}} \ (g/cm^2)$$

Histological and Histochemical Stains: 1 Haematoxylin and Eosin method was used to demonstrate the general histoarchitecture of the liver

2 Masson Trichrome stain was used to demonstrate collagen.

3 Gordon and sweet's silver staining method was used for reticular fibers

Photomicrography: Olympus binocular microscope was used. A 5.1 megapixel MV550 research camera for microscopes was mounted in one of the oculars. This was connected to a computer running on image capture and analysis software. The system was adjusted to obtain clarity and resolution. The image was captured and saved on the computer.

Statistical Analysis: One-way ANOVA was used to analyze data, followed by Student Newman-Keuls test for multiple comparisons. GraphPad Prism 5 (Version 5.03; Graphpad Software Inc., San Diego, CA) was the statistical package to be used for data analysis. Statistically significant difference was set at $p<0.05$.

RESULTS

One way ANOVA revealed that, there was significant difference in weight change across all experimental groups ($F = 7.450$; $p = 0.0079$). Post hoc analysis showed that weight of high fat fed group was significantly lower than the vehicle treated group. The weight reduction induced by high fat fed was significantly and reversed by orlistart administration ($p<0.05$) (Fig. 1). There was significant increase in body mass index of high fat diet group when compared to control group and group C ($F = 7.450$; $p = 0.0079$) (Table 2)

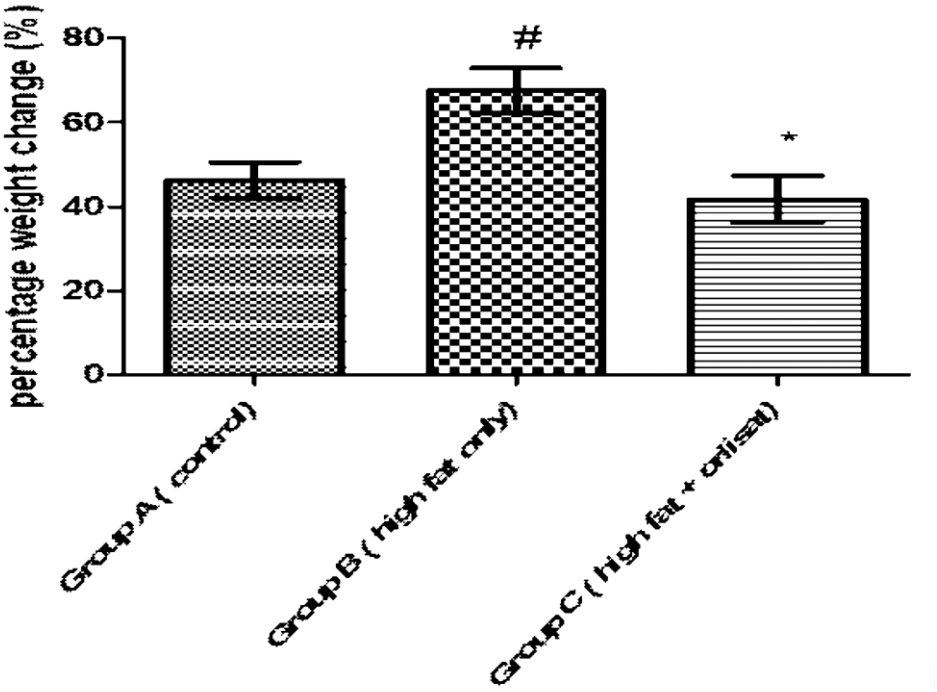

Figure 1: Effect of Orlistst on Body Weight of Rats Fed with High Fat Diet. n = 3, values are expressed as % weight change ± SEM. # = relative to control at $p<0.05$; *= relative to group B (high fat diet) at $p<0.05$.

Groups	Body Mass Index (BMI) (g/cm^2)	
	Baseline	Final
A (Control)	0.50±0.02	0.54±0.02
B (High fat fed)	0.49±0.02	0.80±0.04$^{\#*}$
C (High Fat Fed + Orlistat)	0.54±0.04	0.58±0.03

Table 2: The Effect of Orlistat on Body Mass Index (BMI) of Rats in High Fat Diet. n = 3, Values are expressed as relative heart weight (%) ± SEM, there was significant difference across the groups. # = relative to control at $p<0.05$; *= relative to group B (high fat diet) at $p<0.05$.

Histological findings: H&E revealed that the liver of the control group is healthy and normal as the section is free from collections and inflammatory cell (Fig. 2A). high group only showed necrosis of hepatocytes focal area of hepatic necrosis occupied by leucocytic cells infiltration and the congestion of central vein were seen (Fig. 2B). The groups that were administered with orlistat showed little perivascular and interstitial infilteration of inflammatory cells. orlistat was able to ameliorate the toxic effect of high fat as they were administered together (Fig. 2C).

Normal histoarchitecture of liver tissue was evident with the sinusoids radially arranged. Also sparse distribution of collagen fibers was observed around the central vein (Fig. 3A). More distribution of Collagen was seen around the portal vein, portal triad and the sinusoids of the high fat diet group (Fig. 3B). Collagen fibers of animals in group C showed similar pattern with that of control animals (Fig. 3C).

There was regular distribution of the reticular fibers around the central vein and the lining of the sinusoids of the liver tissue (Fig. 4A). The reticular fibers were observed to be sparsely distributed as shown in fig. 4B. More distribution of the reticular fibers around the central vein, portal vein and lining the sinusoids was evident in fig. 4C.

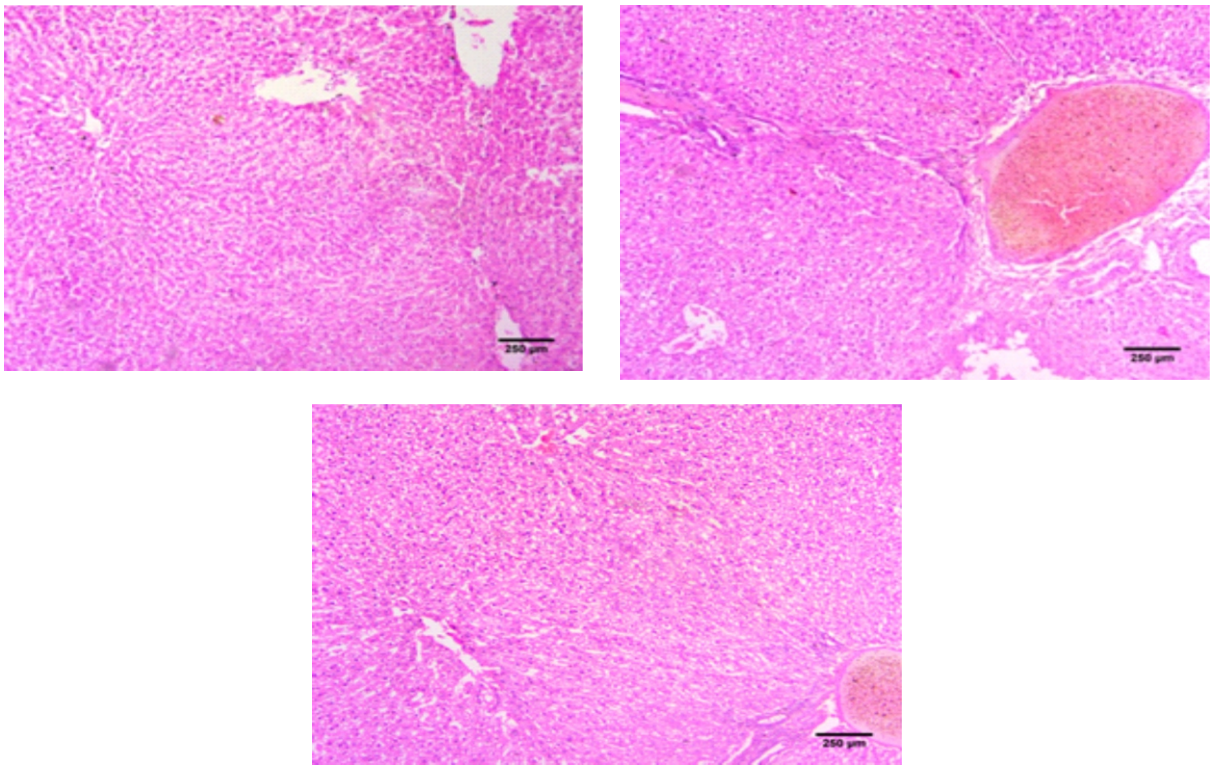

Figure 2: showing the hepatic tissues composed of hepatocytes (H) disposed in sheet, the hepatocytes were seperated by the sinusoids (S). The central vein (CV) was well outlined. Section was free from collections and inflammatory cells in group A (control). The central vein (CV) was congested (CCV) and portal vein (PV). There were necrosis hepatocytes (NP). Section was not free from collections and inflammatory cells in group B (High fat diet only). The central vein (CV) is well outlined and portal vein (PV) in group C (High fat diet + Orlistat). H&E (X 200).

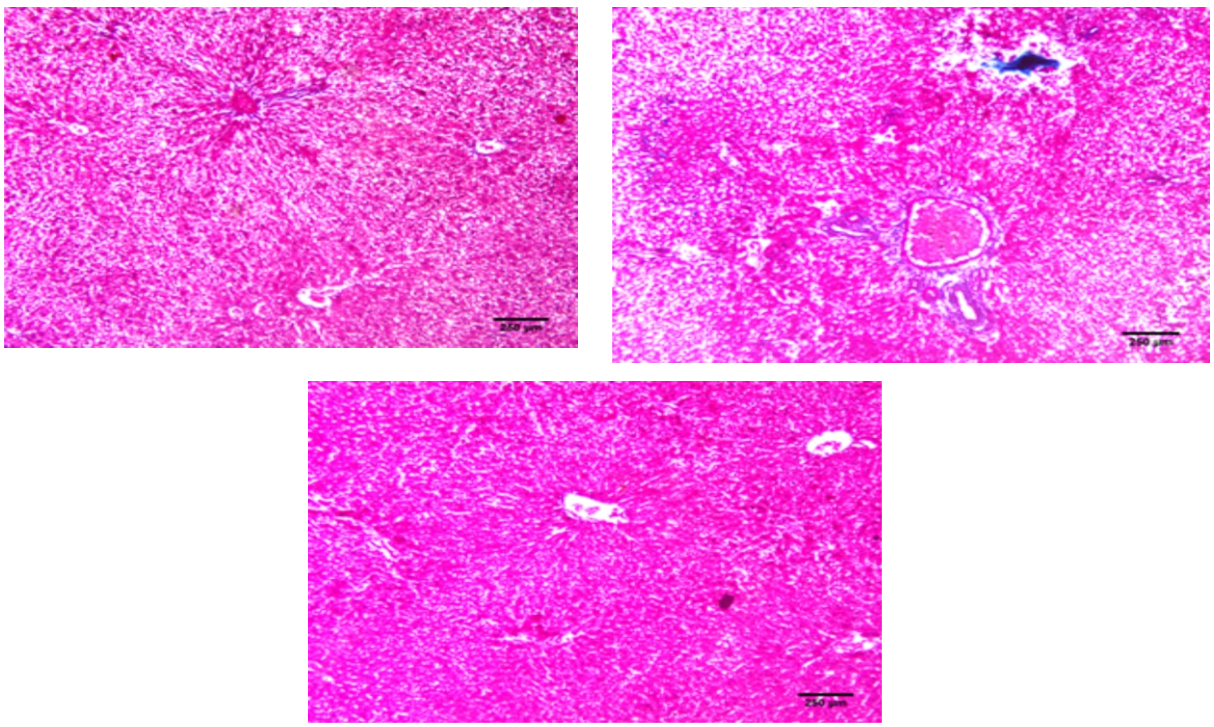

Figure 3: Showing the photomicrographs of Liver of Rats stained with Masson's trichrome stain. There was more collagen fibres (Black arrow) in group B (high fat diet + orlistat) (MT x 200).

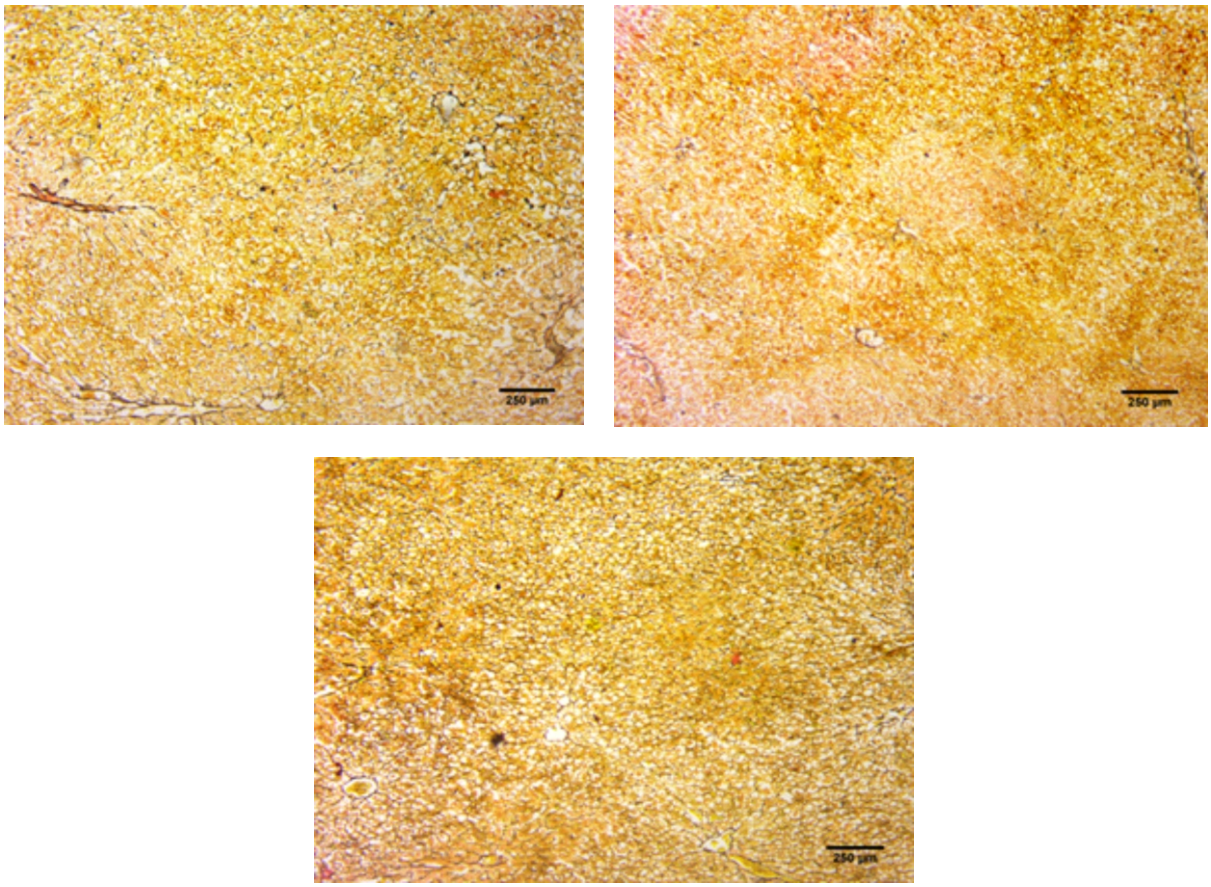

Figure 4: Showing the photomicrographs of Liver of Rats stained with Gordon and Sweets_silver impregnation method (×200). There were sparsely distributions of reticular fibres in group B (high fat diet + orlistat) (Reticular fibers are the black pigment)

DISCUSSION

This study investigated the effects of the treatment of the high fat diet (HFD) with orlistat on the liver of 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 change in the percentage body weight of the experimental animals across the groups, which caused by the high caloric intake in the form of high fat diet increased the body weight, fat content as well as the adiposity index. These results were in accordance with Matos et al.,[6], Rezq & El-Khamisy[7] and also with the report of Samy & Hussein[8]. According to the report of Norshalizah et al., stated that the normal BMI for male Wistar rat was in the range of 0.45 ± 0.02 g/cm^2 to 0.68 ± 0.05 g/cm[29]. In this present study, it showed that high fat diet only had higher BMI of 0.80 g/cm^2 when compared with control and high fat diet + orlistat of 0.54 g/cm^2 and 0.58 g/cm^2 respectively. It has been reported that orlistat is highly efficient when given in conjugation with a high fat diet. It is a reversible lipase inhibitor that acts by inhibiting the absorption of dietary fats[10]. Therefore, Orlistat treatment reduced the body weight, adipse tissues and serum lipids as compared with the HFD group. This could be explained by the reduction in the adipose tissue accumulation[8].

Under microscopic examination, the liver of control animals showed normal cellular architecture and binucleation and was without any distortions. Histology from plate 1B of experimental animals showed a higher extent of tissue damage than those of the control and any other treated experimental group. High fat diet produced changes in the hepatocytes, portal triads and the sinusoids.

In this study, it was observed that reticular fibers dissociated into small fragments in the negative control. Reticular fibers are supporting structures known to form thin and extensive network around the parenchymal cells of the liver within perisinusoidal space[11]. This distortion may be responsible for the distorted architecture observed in group B. According to Junqueira et al.[12] stated that glycosaminoglycan, a ground substance of extracellular matrix, stabilizes reticular fibers. Collagen has a notable function of provision of tensile strength in support tissues.

Mutation of genecoding for collagen has been found to result in reduced tensile strength in support tissues leading to abnormal tissue laxity or susceptibility to injury[11]. The collapsed portal wall, high rate cellular

death and the extension of connective tissue into hepatic parenchyma, in the group B may however be as a result of the depletion of this connective tissue fiber observed in them with the consequent tissue laxity[13].

CONCLUSION

The result obtained from this study showed that high fat diet consumption exhibits adverse effect on the cytoarchitecture of the liver of male Wistar rats. The study also showed that there was a gain in body weight of all animals, suggesting the ability of caffeine consumption to potentiate and accelerate weight increase. Consequently, orlistat treatment reduced the alteration induced by high fat diet.

Conflict of interest

The authors have no conflict of interest to declare.

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