

Protective Effects of Orlistat on Lipid Profile, Cardiac Oxidative Stress Biomarkers and Histology in High-fat Diet-induced Obese Rats

Othman ZA^{a,c}, Wan Ghazali WS^a, Noordin L^a, Omar N^a, Mohd. Yusof NA^b, Mohamed M^{a,d,*}

^aDepartment of Physiology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

^bDepartment of Anatomy, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

^cFaculty of Medicine, Universiti Sultan Zainal Abidin, 20400, Kuala Terengganu, Terengganu, Malaysia

^dUnit of Integrative Medicine, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

ABSTRACT

Introduction: Orlistat is a widely used drug in treating obesity as it promotes weight reduction. The aim of this study was to determine the protective effects of orlistat (10 mg/kg/day) on cardiovascular parameters and oxidative stress biomarkers in high-fat diet (HFD)-induced obese rats. **Methods:** Twenty-four male rats Sprague Dawley rats were divided into three groups and fed with normal diet (N), HFD and HFD with orlistat (HFD+O). Orlistat was administered daily by oral gavage and after six weeks, all rats were sacrificed. **Results:** Administration of orlistat along with HFD (HFD+O) has brought significant decreases in Lee obesity index and LDL level compared to HFD group. Activities of cardiac superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were significantly higher, whereas level of oxidised LDL was significantly lower in HFD+O group compared to HFD group. HFD group had significantly higher necrotic patch area in myocardium while minimal histological changes were seen in HFD+O group. **Conclusion:** This study may suggest that administration of orlistat at 10 mg/kg/day for 6 weeks may have protective effects against the changes on Lee obesity index, lipid profiles, cardiac oxidative stress biomarkers and histology of myocardium in HFD-induced obese rats possibly through its hypolipidaemic and antioxidant actions.

KEYWORDS: orlistat, obesity, cardiovascular, lipid profile, oxidative stress

INTRODUCTION

Obesity has been declared as a global public health concern and it has been documented over 115 million people in the world are suffering from obesity-related problems.¹ The global prevalence of obesity is tremendously worrying as obesity is projected to rise from 396 million in 2005 to 573 million in 2030.² Obesity is closely related to cardiovascular risk diseases such as hyperlipidemia, hypertension and coronary artery disease.³

Previous study has shown that administration of high-fat diet (HFD) for 14 weeks successively induced obesity and alters normal morphological function of heart and liver in male Sprague-Dawley rats due to lipid accumulation process.⁴

Pathogenesis of obesity-related disease is believed to be as a consequence of abundance of free radicals which can react with macromolecules in animal or human by affecting proteins, carbohydrates, lipid, and DNA sequence, which subsequently debilitate the normal cell function.^{5,6} Presence of naturally defence antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have been shown to combat oxidative stress by reducing lipid peroxidation process shown by low malondialdehyde (MDA) level in HFD-induced obese rats.⁷

Corresponding Author:
Assoc. Prof. Dr. Mahaneem Mohamed,
Department of Physiology,
School of Medical Sciences,
Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan, Malaysia.
Tel No : +6097676158
Email: mahaneem@usm.my

Pharmacological approach is one of the adjunct therapies applied by health professionals other than dietary and lifestyle changes. Orlistat is a non-centrally acting drug and has been widely used as it reduces 30 % of dietary fat absorption by inhibiting gastropancreatic lipase in the stomach and intestine, respectively.⁸ It is applicable for patients with body mass index (BMI) of more than 40 kg/m² and those who suffered from ≥ 2 obesity-related medical conditions including coronary heart disease, type 2 diabetes mellitus, hypertension, dyslipidemia, cerebrovascular disease, severe osteoarthritis and sleep apnea.⁹ Orlistat has been shown to reduce total cholesterol and low-density lipoprotein (LDL)¹⁰ and its side effects include oily fecal, diarrhoea, increased flatulence and abdominal discomfort.¹¹

Significant reduced BMI and increased serum MDA level are observed in obese patients treated with orlistat for 6 months.¹² Orlistat treatment for 6 weeks also significantly reduces liver MDA level and increases liver SOD, GPx and CAT activities in rats fed with HFD compared to control group.¹³ In addition, treatment of orlistat induces significant weight loss and improvements in left ventricular diastolic function and in sympathovagal balance among severely obese subjects with diabetes.¹⁴

However, no study has been reported on the protective effect of orlistat against cardiac oxidant-antioxidant status and the histology of myocardium in obesity. Therefore, this study aimed to determine the effects of orlistat administration on some cardiovascular parameters such as Lee obesity index, total food and calorie intake, lipid profile, cardiac oxidant-antioxidant status and histology of myocardium in HFD-induced obese rats.

MATERIALS AND METHODS

Materials

Orlistat was purchased from Aslene, Malaysia. Hematoxylin, eosin and formaldehyde were purchased from Richard-Allan Scientific (USA), Sigma-Aldrich (USA) and Merck (Germany), respectively. Cholesterol powder was purchased from Nacalai Tesque (Japan). Assay kits for CAT, glutathione peroxidase (GPx), SOD and total protein were purchased from EnzyChrom™ (USA). Assay kits for malonaldehyde and oxidized LDL (oxLDL) were purchased from Northwest Life Sciences (USA) and

Cloud Corne (USA), respectively.

Normal and high-fat diet (HFD)

Normal chow diet comprised of standard Altromin pellet, which was purchased from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia (USM). The HFD regime was prepared by mixing 64 g of normal chow, 32 g of saturated fat from animal (ghee), 300 IU vitamin D₃¹⁵ and 12 % of cholesterol powder. The mixture was shaped into hand-ball size before it was stored in a fridge at 4 °C. HFD was prepared every other day to protect lipid from oxidation process. Normal chow diet contained 12 % of fat, 24 % of protein, and 64 % of carbohydrate (total energy was 3.19 kcal/g) while HFD contained 31.45 % of fat, 11.59 % of protein and 46.31 % of carbohydrate (total energy was 5.17 kcal/g).

Experimental design

Twenty-four male Sprague-Dawley rats (8-10 weeks old, weighing 180-230 g) were housed in a well-ventilated facility with 12-h light/dark cycle at 22-24 °C. After acclimatization, rats were assigned into three groups (n=8) i.e. Normal group (N) which received normal chow diet *ad libitum*, HFD group which received HFD alone, and HFD+O group which received HFD and orlistat at 10 mg/kg/day (HFD+O) based on previous study.¹⁶ Body weight and food intake were measured every two days and daily, respectively.

All experimental protocols were performed according to guidelines from Animal and Research Centre, Universiti Sains Malaysia, Kelantan [USM/Animal Ethics Approval / 2016 / (98) (744)]. Following 6 weeks period, Lee obesity index was calculated and the rats were anaesthetized with 90 mg/kg ketamine and 5 mg/kg xylazine. Laparotomy was done to collect the blood from posterior vena cava and to harvest the heart.

Measurement of Lee obesity index

Lee obesity index was determined in this animal study. It is an index to confirm obesity whereby its value of more than 315 is considered as obese.¹⁷ It was calculated based on the formulae described by previous study¹⁸ whereby cube root of body weight (g) was divided by nose-to-anus length (cm) of the rat.

Measurement of serum lipid profile

The blood sample was allowed to coagulate before being centrifuged. Serum was collected for measurement for lipid profile. Total cholesterol was determined by an enzymatic-colorimetric method using Architect c Cholesterol Reagent kit. The indicator quinoneimine was produced from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Triglyceride was determined by enzymatic hydrolysis using Architect c Triglyceride Reagent kit which converts triglyceride to free fatty acids and glycerol. Determination of high-density lipoprotein (HDL) involved elimination of chylomicron, LDL and very low-density lipoprotein by cholesterol esterase, cholesterol oxidase and catalase using Biosino Direct HDL-Cholesterol reagent kit. Measurement of LDL was determined by Friedwald formula as below:¹⁹

$LDL \text{ (mmol/L)} = \text{Total cholesterol} - HDL + \text{Triglyceride}/5$.

Biochemical measurement

The heart tissue was homogenized in 10% ice-cold phosphate buffer solution before being centrifuged. The supernatant was collected and used to determine activities of SOD, GPx, and CAT. Total protein content of samples were used to express the activity of antioxidant enzymes. Levels of oxLDL and MDA were also determined.

Histology of myocardium

The heart tissue was dissected out, rinsed immediately with phosphate buffer solution and fixed in 10% formalin solution. Following fixation, the heart section was embedded in a paraffin block, sliced using microtome machine at 5 μm size, and stained with Haematoxylin and Eosin solution.²⁰

Statistical analysis

All data are expressed as mean (standard deviation). Prior to statistical analysis, all variables were checked for normal and homogeneous variance using the Levene test. The mean differences were statistically analyzed by performing one-way analysis of variance (ANOVA) test with Tukey post-hoc test for multiple comparisons. *P* value < 0.05 was considered as statistically significant.

RESULTS

Effects of orlistat on Lee obesity index, weight gain, mean food intake, and calorie intake.

HFD group had significantly higher Lee obesity index and calorie intake compared to N group. Meanwhile, HFD+O group had significantly lower Lee obesity index compared to HFD group and had significantly higher total calorie intake compared to N group. No significant differences were found for weight gain and food intake among all groups (Table 1).

Table 1. Effects of orlistat on Lee obesity index, weight gain, mean food intake and calorie intake in all groups.

	N	HFD	HFD+O
Lee obesity index	310.94 (2.69)	339.87 (12.82) ^a	320.51 (12.60) ^b
Weight gain (g)	98.58 (39.28)	120.09 (22.80)	105.90 (22.57)
Mean Food intake (g/day)	21.11 (2.68)	18.43 (1.28)	20.97 (0.96)
Calorie intake (Kcal/day)	67.13 (3.72)	94.91 (6.59) ^a	107.97 (4.95) ^a

Data are presented in mean (standard deviation), n=8 per group.

^a *P* < 0.05 compared with normal group.

^b *P* < 0.05 compared with HFD group.

One-way ANOVA followed by Tukey post-hoc test.

N; normal, HFD; high-fat diet, HFD+O; high-fat diet and orlistat.

Effects of orlistat on lipid profile

Both total cholesterol and LDL levels were significantly higher in HFD group compared to N group. In HFD+O group, LDL level was significantly lower when compared to HFD group. However, no significant differences were observed on serum triglyceride and HDL levels among all groups (Table 2).

Table 2. Effects of orlistat on serum lipid profile in all groups.

	N	HFD	HFD+O
Total Cholesterol (mmol/L)	1.76 (0.19)	2.29 (0.20) ^a	1.90 (0.26) ^b
Triglyceride (mmol/L)	0.79 (0.32)	1.09 (0.35)	1.03 (0.36)
LDL (mmol/L)	0.20 (0.17)	0.41 (0.22) ^a	0.16 (0.10) ^b
HDL (mmol/L)	1.19 (0.16)	1.23 (0.12)	1.31 (0.16)

Data are presented in mean (standard deviation), n=8 per group.

^a *P* < 0.05 compared with normal group.

^b *P* < 0.05 compared with HFD group.

One-way ANOVA followed by Tukey post-hoc test.

N; normal, HFD; high-fat diet, HFD+O; high-fat diet and 10 mg/kg/day orlistat. LDL; low-density lipoprotein. HDL; high-density lipoprotein.

Effects of orlistat on oxidant-antioxidant status

SOD activity was significantly lower and MDA level was significantly higher in HFD group compared to N group. Meanwhile, HFD+O group had significantly higher SOD, GPx and CAT activities, and lower oxLDL level compared to HFD group (Table 3).

Table 3. Effects of orlistat on cardiac oxidant-antioxidant status in all groups

	N	HFD	HFD+O
Catalase (U/mg protein)	1.03 (0.29)	0.66 (0.27)	1.23(0.33) _b
Glutathione peroxidase (U/mg protein)	435.69 (19.00)	431.58 (3.50)	566.66 (16.19) ^{a,b}
Superoxide dismutase (U/mg protein)	2.55 (0.69)	1.55 (0.34) ^a	2.47(0.54) _b
Malondialdehyde (nmol/mg protein)	0.17 (0.02)	0.24 (0.05) ^a	0.19(0.46)
Oxidised LDL (pg/mL)	261.79 (49.85)	304.27 (66.32)	210.00 (27.01) _b

Data are presented in mean (standard deviation), n=8 per group.

^a P < 0.05 compared with normal group.

^b P < 0.05 compared with HFD group .

One-way ANOVA followed by Tukey post-hoc test.

N; normal, HFD; high-fat diet, HFD+O; high-fat diet and 10 mg/kg/day orlistat.

Effects of orlistat histology of myocardium

Histological appearance of myocardium tissue showed the presence of significantly large necrotic patch area in HFD group compared to N group. However, in HFD+O group, these changes were minimally seen (Figure 1 and Table 4).

Table 4. Quantitative analysis of necrotic area in all groups

	N	HFD	HFD+O
Necrotic area (mm ²)	0.00(0.00)	0.54(0.14) ^a	0.00(0.00) _b

Orlistat significantly reduced necrotic area in myocardial tissue of rats fed with HFD. Data are presented in mean (standard deviation). n=8.

^a P < 0.05 compared with normal group.

^b P < 0.05 compared with HFD group.

One-way ANOVA followed by Tukey post-hoc test.

N; normal, HFD; high-fat diet, HFD+O; high-fat diet and 10 mg/kg/day orlistat.

DISCUSSION

There were some cardiovascular changes in male Sprague-Dawley rats following HFD ingestion for 6 weeks. Although, there was no significant changes in body weight gain and food intake, HFD regime used in the present study was able to establish a successful animal model of obesity as shown by the

significantly higher Lee obesity index in HFD group compared to N group. Meanwhile, Lee obesity index was significantly lowered in HFD+O group compared to HFD group which might indicate some protective effect of orlistat against obesity. Mean calorie intake in group administered with HFD (HFD and HFD+O groups) were significantly higher compared to N group which is similar to previous study.²¹ The increased total calorie intake in these HFD groups were attributed to the higher content of fat composition in HFD regime.

After 6 weeks of HFD administration, total cholesterol and LDL levels were significantly higher in HFD groups compared to N group. This could be due to ingestion of higher fat content in HFD food regime used in the present study that subsequently might increase lipid metabolism pathway. Orlistat had significantly lowered levels of total cholesterol and LDL as shown in HFD+O group. This finding could be due to effect of orlistat which caused excessive fecal fat loss as a result of inhibition of fat absorption in HFD-induced obese rats.²² On the other hand, both HFD and HFD+O groups had insignificant changes on the levels of TG and HDL which are consistent with previous study.²³

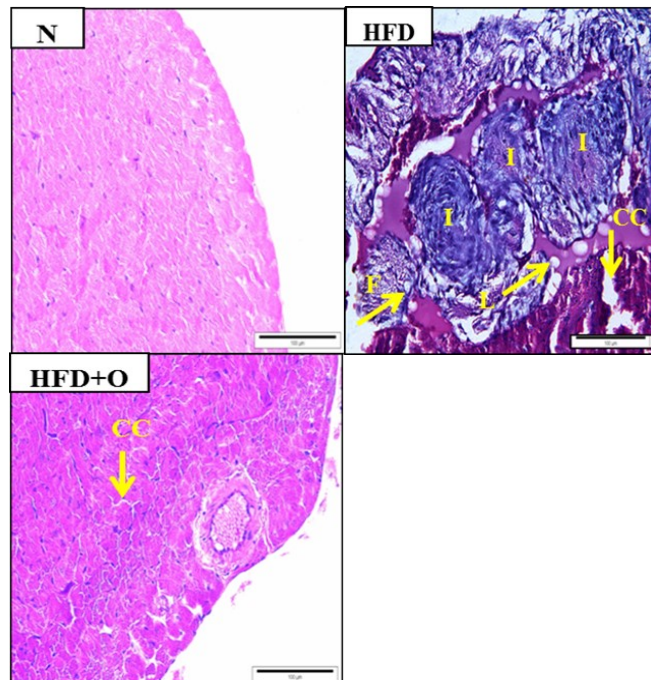


Figure 1. Histopathological sections of myocardium stained with H&E in all groups. N group shows normal appearance of myocardial tissue with no pathological changes. HFD group shows presence of necrotic patch which consists of foam cells (F), inflammatory cells (I), lipid droplets (L) and wide cholesterol cleft (CC). HFD+O group shows the presence of cholesterol cleft in between the myocardial fibers. N, normal; HFD, high fat diet; HFD+O, HFD and 10 mg/kg orlistat. (Haematoxylin and Eosin staining, magnification X100, scale bar=100µm).

Fatty acids which are the building block for lipid are susceptible to oxidation process partly through the presence of free radicals as intermediate.²⁴ In the present study, HFD group had higher levels of cardiac MDA compared to N group suggesting the presence of oxidative stress in the heart following HFD intake. This oxidative stress is contributed by HFD due to the generation of free radicals which further leads to formation of lipid peroxidation.²⁵ Involvement of lipid peroxidation product shows an evidence of the presence of toxic radicals as a consequence of obesity.²⁶ However, the level of cardiac MDA in HFD+O group was not significantly different from N group, which might suggest some protective effect against lipid peroxidation. In addition, oxLDL was significantly lower in HFD+O group than HFD group which might further suggest that orlistat is able to reduce cardiac oxidative stress level.

HFD group had significantly lowered SOD activity compared to N group presumably due to the presence of cardiac oxidative stress. Administration of orlistat for 6 weeks duration revealed significant increases of SOD, GPx and CAT activities compared to HFD group which are consistent with another study on the liver.¹³ The findings in the present study might suggest the antioxidant effect of orlistat against oxidative stress in rats fed with HFD. HFD group showed the presence of large necrotic patch area which was significantly larger compared to N group. On the other hand, HFD+O group showed an improvement on histological changes of myocardium tissue which showed significant smaller necrotic patch area compared to HFD group as well as higher SOD, GPx and CAT activities in HFD+O group. Several studies have proposed that inflammation is derived from accumulation of lipid in the myocardium of HFD-fed rats.²⁷ However, it is not known whether orlistat may also improve inflammation in obesity which needs further study to determine its exact mechanism of action. The improved histological finding in HFD+O group could be due to the protective effect of orlistat against oxidative stress as shown by lower levels of oxLDL and MDA.

CONCLUSION

The findings of the study may suggest that administration of orlistat at 10 mg/kg/day for 6 weeks had protective effects on Lee obesity index, lipid profile, cardiac oxidant-antioxidant status and

histology of myocardial tissue in HFD-induced obese rats. These effects might be possibly due to its hypolipidemic and antioxidant effects which need further study to understand its exact mechanism of action.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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