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Original Article

TO STUDY THE ROLE OF EXERCISE AND DIETARY MANIPULATION IN OBESE RATS TREATED WITH ORLISTAT

SARATH CHANDRA M[1], JYOTHI. Y[2]*, SYED IMAM RABBANI[3]

[1,2,3]**Krupanidhi College Of Pharmacy, No 12/1, Carmelaram Road, Chikka Bellandur, Carmelaram Post, Varthur Hobli, Bengaluru, Karnataka 560035**

***Email: jokiran05@gmail.com**

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ABSTRACT

Objective: The present study was undertaken to evaluate the role of exercise and dietary manipulation in obese rats treated with Orlistat.

Methods: The study was conducted for 16 weeks. High-fat diet (HFD) was used to induce obesity, and once these animals were obese (eight week), manipulation in the diet, exercise, Orlistat and low dose of Orlistat were tested for 8 more weeks. During the treatment period, animals were continued with HFD except for the groups where the low-calorie diet (LCD) was used. The various manipulative options studied to include swimming test (physical exercise), Orlistat (200 mg/kg), low dose Orlistat (65 mg/kg) and low-calorie diet. The parameters were evaluated including Body weight was measured on weekly two days. Biochemical parameters such as HDL, LDL, VLDL, Triglycerides and total cholesterol were measured using diagnostic kits. Blood-glucose level was measured. On 16th week, the animals were sacrificed and their organ weights were measured. Organ weights included the Liver, Left and Right kidney and fat pad weights such as Mesenteric fat, Epididymis fat.

Results: There was a significant reduction in body weight, Triglycerides, total cholesterol, LDL, VLDL, Blood-glucose level organ weights included the Liver, Left kidney and Right kidney and fat pad weights such as Mesenteric fat, Epididymis fat and increased in HDL level of the animals treated with exercise and dietary manipulation and Orlistat.

Conclusion: The present study revealed that [HFD+Orlistat (low dose)+Exercise+LCD] group showed more potential effective than rest of all treatment groups.

Keywords: Anti-obesity, High-fat diet, Low-calorie diet, Exercise, Orlistat.

INTRODUCTION

Obesity is a medical condition in which excess body fat gets accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Among the multiple factors contributing to its etiology, the sedentary life styles, white-collar jobs, lack of exercise, psychological factors, and the consumption of energy-rich diets are the major ones [1, 2].

Due to obscure etiology, the treatment of obesity is difficult and challenging. Further, the cause of concern is the non-availability of drugs for its treatment and the short-term efficacy and limiting side effects of the available drug [3].

It is the accumulation of body fat to the level which might have a negative effect on health. Body Mass Index (BMI) is a widely used diagnostic tool which provides a simple numeric measure of people 'fatness'. BMI of 18.5 to 25 may indicate optimal weight a number above 25 may indicate the person is overweight; while a number above 30 suggests the person is obese and over 40, indicates morbidly obese. Indian urban population is experiencing high rates of obesity, as their work often demands less physical exertion. Even rural areas are not immune because of increased mechanization of farming activity leading to reduced physical activity [4].

The prevalence of obesity is increasing worldwide. Numerous diseases are caused or made worse by obesity. These include type two diabetes; hypertension; dyslipidemia; ischemic heart disease; stroke; obstructive sleep apnea; asthma; non alcoholic steato hepatitis; gastro esophageal reflux disease; degenerative joint disease of the back, hips, knees, and feet; infertility and poly cystic ovary syndrome; various malignancies; and depression [5].

A compound that selectively limits the intestinal absorption of dietary fat, in excess of that manageable by dietary manipulation alone, could become a useful therapeutic agent for the treatment of obesity. The inhibition of gastrointestinal lipase may be a potential method for decreasing fat absorption. Pancreatic lipase is the key enzyme required for the absorption of dietary triglyceride. Pancreatic lipase may also be indirectly responsible for an absorption of cholesterol.

Orlistat is a pharmacological agent that promotes weight loss in obese subjects via inhibiting of gastric and pancreatic lipase, an enzyme that is crucial for the digestion of the long-chain triglycerides, which at three daily doses of 120 mg reduces fat absorption by 30% and has been proven to be useful in facilitating both weight loss and weight maintenance [6].

However, because Orlistat can result in undesirable side effects, such as fecal incontinence, flatulence, and steatorrhea, its use may be limited. Therefore, it may be worthwhile to search the natural substances that show potent inhibitory activity against pancreatic lipase and have fewer side effects [7].

Increasing dietary fat increases body fat and it is unlikely that humans escape this important biological rule. Increasing dietary fat is associated with increased prevalence of obesity probably by increasing the intake of energy dense foods.

In the National Weight Loss Registry, three things were associated with weight loss: continued monitoring of food intake, lowering dietary fat intake, and increased exercise. The relation of dietary fat is most evident when physical activity is low [8].

Physical exercise can be used effectively by itself or in combination with dietary restriction to trigger weight loss [9]. Hence, the present study is designed to evaluate the effect of dietary manipulation and exercise in the management of obesity in Orlistat treated rats.

MATERIALS AND METHODS

Chemicals and reagents

Triglyceride and Cholesterol Kit (Span Diagnostics, Bangalore, India), Orlistat Capsules 120 mg (Torrent Pharmaceuticals LTD,

India). All other reagents used in the experiments were of analytical grade and of the high purity.

Table 1: Composition of experimental diets

Composition of diet	High-fat diet (HFD) in gm	Low-calorie diet LCD) in gm
1. Fat:		
a. Lard	230	0
b. Vegetable oil	50	45
2. Carbohydrate:		
a. Dextrin	97	95
b. Cornstarch	98	95
c. Sucrose	130	445
3. Protein:		
a. Casein	325	250
4. Vitamin mix	30	30
5. Mineral mix	40	40
6. total weight(g)	1000	1000
7. Energy density(k cals)	5.15	3.93
8. % Macronutrient(k cals)		
a. Fat	50	10
b. Carbohydrate	25	65
c. protein	25	25

Ingredients expressed by weight (gm), HFD (high-fat diet); LCD (low-calorie diet)

Description of diet [10]

Animals

Female Sprague-Dawley rats were obtained from the Central Animal house, Krupanidhi College of Pharmacy, Bangalore, India. The rats were housed under 22±2ºC temperatures, 40-60% humidity and 12-12±1 h light-dark cycle. During obesity induction, rats weighing 150-200g were taken for the study, and they were broadly divided into ten groups. Group one was normal control (Normal pellet diet was fed) and nine groups were fed with HFD (High-fat diet) to induce obesity and water *ad libitum*. Experimental protocols were followed as per Institutional Animal Ethical committee guidelines. Animal Ethical committee clearance (CPCSEA No. KCP/IAEC-005/2013-14) was obtained for the procurement of animals.

Experimental design

The SD female rats were divided into 10 major groups. Each of the subgroups consists of six animals as follows:

1. Normal Control-Rats will be fed on normal pellet diet
2. HFD Control-Rats will be fed on high-fat diet [11]
3. HFD+Exercise [12]
4. HFD+Low-calorie diet-(Obese rats will be switched from HFD to LCD) [11]
5. HFD+LCD+Exercise
6. HFD+Orlistat (200 mg/kg diet) [13]
7. HFD+Orlistat (low dose) (100 mg/kg)
8. HFD+Orlistat (low dose)+Exercise
9. HFD+Orlistat (low dose)+LCD
10. HFD+Orlistat (low dose)+LCD+Exercise

Exercise protocols [12]

Swimming

The rats will be adapted to the water before the training began. The adaptation will consist of swimming for 30 minutes, once per day for five days, in water at a temperature of 31 °C. After adaptation, the rats will be trained by swimming for 60 minutes/day, five days a week for eight weeks, with a constant overload equivalent to 5% of their body weight. The water tanks will be 50 cm in height and 30 cm in diameter, and the over load (lead fish sinkers) was attached to the animal's chest using an appropriate vest.

Measurement of body weight

Body weight was measured on every alternative day for 16 weeks.

Estimation of plasma lipid profiles

At the end of the experiment, on 16th-week blood samples were collected from overnight fasted animals under inhalation of anesthesia by retro-orbital puncture method. Plasma was separated by centrifugation at 2500 rpm for 15 min and was used for further experiments, i.e. Total cholesterol, HDL and triglyceride levels were estimated by CHOD-PAP method and GPO-PAP method. LDL levels were calculated by the method of Johnson *et al.*[14]. Blood glucose is estimated by using Glucometer.

Estimation of organ weight and fat pad weight

On 16th-week the animals were sacrificed by cervical dislocation and organs like Liver and kidney weights has taken. Fat pads like Mesenteric and epididymal fat are collected and dried on filter paper and weighed.

Statistical analysis

The results will be expressed as mean±SEM. Comparisons between the treatment groups and control will be performed by analysis of variance (ANOVA) followed by Turkeys' multiple range tests. In all tests, the criterion for statistical significance will be ($p<0.05$).

RESULTS

Body weight

High-Fat Diet substantially increased body weights of rats in 16th-week, when compared to the normal control group. On 16th-week, when compared to HFD control, all Treatment groups showed a significant decrease in body weight. [HFD+Orlistat (low dose)+Exercise+LCD] group showed more potential effective than rest of all treatment groups (table 2).

Table 2: Effect of exercise and dietary manipulation on body weight

S. No.	Experiment groups	1 Week	8 Week	16 Week
1	Normal control	150±1.91	200.16±3.24	260.83±2.7
2	HFD (high-fat diet) control	153.3±2.94	250.33±3.16^{a}	350.5±4.52^{a}
3	HFD+Exercise	152.17±3.1	253.67±3.40^{a}	330±3.21^{a,x}
4	HFD+LCD (low-calorie diet)	154.67±2.64	252.83±4.54^{a}	345.5±3.62^{a}
5	HFD+LCD+Exercise	151.5±3.15	255.67±3.20^{a}	325±2.83^{a,x}
6	HFD+Orlistat	150.17±1.85	248±2.42^{a}	335.83±2.97^{a,x}
7	HFD+Orlistat (low dose)	153.3±2.60	251.67±3.92^{a}	342.17±3.15^{a,y}
8	HFD+Orlistat (low dose)+Exercise	150.3±3.09	254.17±4.35^{a}	322.33±2.53^{a,x}
9	HFD+Orlistat (low dose)+LCD	152.67±3.05	250.83±2.74^{a}	339±2.97^{a,y}
10	HFD+Orlistat (low dose)+LCD+Exercise	154.17±2.37	253±2.11^{a}	318.33±4.11^{a,x}

All values are mean±SEM. $^{a}P<0.001$, $^{b}P<0.01$, $^{c}P<0.05$ When compared to the normal control group, $^{x}P<0.001$, $^{y}p<0.01$, $^{z}p<0.05$ When compared to HFD control.

Table 3: Effect of exercise and dietary manipulation on bio chemical parameters

S. No.	Experiment groups	Triglycerides	Cholesterol	HDL	LDL	VLDL	Blood glucose
1	Normal control	96±2.07	67.50±1.84	50.3±2.19	35.3±1.31	20.67±0.8	100±1.63
2	HFD (high-fat diet) control	151.17±1.96[a]	143.17±2.14[a]	25.3±1.38[a]	60.5±0.76[a]	35.5±2.78[a]	150.1±2.02[a]
3	HFD+Exercise	130.8±2.41[a,x]	118.5±1.09[a,x]	37.5±1.87[a,y]	46.17±1.87[a]	25.5±1.95[z]	133±2.8[a,x]
4	HFD+LCD (low-calorie diet)	145.83±2.73[b]	138.17±1.01[a]	30.3±1.72[a]	55.17±1.51[a]	32.17±1.6[b]	145.5±1.95[a]
5	HFD+LCD+Exercise	125.17±1.6[a,x]	116.17±2.5[a,x]	38.6±0.84[b,x]	47.83±1.78[a,x]	24.3±1.5[y]	128.8±1.87[a,x]
6	HFD+Orlistat	135.8±2.75[a,y]	121±1.37[a,x]	36.3±2.23[a,y]	43.17±2.3[x]	26.6±1.97[z]	137.1±1.8[a,y]
7	HFD+Orlistat (low dose)	143.67±2.03[a]	135±1.24[a,z]	31.6±2.47[a]	53±2.19[a]	31±1.59[b]	144±1.57[a]
8	HFD+Orlistat (low dose)+Exercise	109.6±3.28[a,x]	111±1.50[a,x]	41.16±0.94[c,x]	39±1.65[x]	19.5±1.54[x]	126.8±1.6[a,x]
9	HFD+Orlistat (low dose)+LCD	140±2.16[a]	133.67±1.9[a,y]	35.3±1.6[a,y]	50±1.63[b,x]	30.8±2.57[c]	141.6±1.89[a]
10	HFD+Orlistat (low dose)+LCD+Exercise	105.3±4.41[x]	100.50±1.84[a,x]	45.83±2.03[x]	29.67±1.43[x]	18.3±1.28[x]	123.3±2.7[a,x]

All values are mean±SEM [a]P<0.001, [b]P<0.01, [c]P<0.05 When compared to the normal control group, [x]P<0.001, [y]p<0.01, [z]p<0.05 When compared to HFD control.

Table 4: Effect of exercise and dietary manipulation on organ fat pad weight

S. No.	Experiment groups	Epididymal Fat (gm)	Mesentric Fat (gm)
1	Normal control	3.27±0.27	2.56±0.21
2	HFD (high-fat diet) control	8.25±0.23[a]	7.31±0.27[a]
3	HFD+Exercise	4.69±0.50[x]	4.94±0.29[a,x]
4	HFD+LCD (low-calorie diet)	7.57±0.22[a]	6.95±0.18[a]
5	HFD+LCD+Exercise	4.52±0.23[x]	4.56±0.32[a,x]
6	HFD+Orlistat	5.21±0.17 [b,x]	5.24±0.24[a,x]
7	HFD+Orlistat (low dose)	7.34±0.33[a]	6.23±0.23[a]
8	HFD+Orlistat (low dose)+Exercise	3.74±0.48[x]	4.41±0.23 [a,x]
9	HFD+Orlistat (low dose)+LCD	6.41±0.32[a,y]	5.65±0.22[a,y]
10	HFD+Orlistat (low dose)+LCD+Exercise	3.7±0.38 [x]	4.35±0.38[a,x]

All values are mean±SEM [a]P<0.001, [b]P<0.01, [c]P<0.05 When compared to the normal control group, [x]P<0.001, [y]p<0.01, [z]p<0.05 When compared to HFD control.

Table 5: Effect of exercise and dietary manipulation on organ weight

S. No.	Experiment groups	Right kidney WT (g)	Left kidney WT (g)	Liver WT (g)
1	Normal control	0.75±0.04	0.73±0.03	6.03±0.33
2	HFD (high-fat diet) control	1.65±0.04[a]	1.62±0.05[a]	11.3±0.3[a]
3	HFD+Exercise	1.22±0.03[a,x]	1.30±0.03[a,x]	9.63±0.56[a]
4	HFD+LCD (low-calorie diet)	1.42±0.03 [a,y]	1.48±0.04[a]	10.65±0.39[a]
5	HFD+LCD+Exercise	1.05±0.04[a,x]	1.20±0.06[a,x]	9.35±0.59[a,z]
6	HFD+Orlistat	1.29±0.03[a,x]	1.33±0.03[a,y]	10.03±0.27[a]
7	HFD+Orlistat (low dose)	1.41±0.02[a,y]	1.45±0.03 [a]	10.33±0.26[a]
8	HFD+Orlistat (low dose)+Exercise	1.02±0.06 [a,x]	0.26±0.07[a,x]	9.28±0.37[a,x]
9	HFD+Orlistat (low dose)+LCD	1.35±0.07[a,x]	1.34±0.03[a,y]	10.22±0.34[a]
10	HFD+Orlistat (low dose)+LCD+Exercise	1.15±0.04[a,x]	1.18±0.05[a,x]	923±0.4[a,x]

All values are mean±SEM. [a]P<0.001, [b]P<0.01, [c]P<0.05 When compared to the normal control group, [x]P<0.001, [y]p<0.01, [z]p<0.05 When compared to HFD control.

Serum lipid profiles

Rats fed with HFD showed increased levels of serum Triglycerides, LDL, VLDL and total cholesterol and decreased HDL levels, when compared normal control. All Treatments groups showed significantly suppressed the rise of Lipid profile and rise in the HDL levels was observed. [HFD+Orlistat (low dose)+Exercise+LCD] group showed more potential effective than rest of all treatment groups (table 3).

Blood glucose

Treatment groups showed a significant decrease in blood-glucose levels when compared to the HFD control group. [HFD+Orlistat (low dose)+Exercise+LCD] group showed more potential effective than rest of all treatment groups (table 3).

Organ weight and Fat pad analysis

Treatment groups showed a significant activity in decreasing the organ weight and Fat pad weights [HFD+Orlistat (low dose)+Exercise+LCD] group showed more potential effective than rest of all treatment groups (table 4 and 5).

DISCUSSION

The present study was designed to evaluate the role of exercise and dietary manipulation on the markers of obesity in rats. The study was conducted for 16 weeks High-fat diet (HFD) was used to induce obesity and once the animal, were obese (eight week), manipulation in diet, exercise, dose of Orlistat was tested for 8 more weeks. During the treatment period animals were continued with HFD except for the groups where the low-calorie diet (LCD) was used. The various manipulative options studied to include swimming test (physical exercise), low dose Orlistat (65 mg/kg) and low-calorie diet.

The parameters evaluated in this include body weight, biochemical parameters such as HDL, LDL, VLDL, triglycerides and total cholesterol measured using diagnostic kits, blood-glucose level measured by using glucometer. On 16th week, the animals were sacrificed and their organ weights were measured, such as the liver, left kidney and right kidney and fat pad weights such mesenteric fat, epididymis fat.

Obesity is a metabolic disorder characterized by an excessive accumulation of fat in the body to an extent which adversely affects the health of an individual. It is a direct consequence of perpetual

imbalance between energy intake and expenditure with storage of extra calories in the form of fat in the adipose tissue. In obesity, there is an increase in intake of high-fat and high-energy food and a decrease in daily energy expenditure [4].

Diet and physical exercise remain as the main stay in obesity management; nonetheless, anti-obesity drugs may be required either to reduce appetite or to inhibit fat absorption. Various factors are found to be involved in obesity, including the genetic, environmental, behavioral, and socio-economic factors.

The present study indicated that administration of HFD to the animals for eight weeks significantly (P<0.001) increased body weight, LDL, TG, VLDL, blood-glucose level, weights of liver, left kidney, right kidney, mesenteric fat, epididymis fat and reduced the HDL level compared to normal control (table 1-4). HFD is a standard diet widely used experimentally to induce obesity in animals. The composition of HFD contains pork fat, glucose, casein, Nacl, dextrin. Earlier research indicated that expose of these ingredients to the experimental animals alters the metabolic mechanisms resulting in obesity. The data indicated that HFD induced obesity is due to hyperlipidemia, and hyperglycemia were evidence by a gain in body weight, increase in triglyceride levels and as well as increasing in the glucose levels [15]. Our observation also indicates that HFD administration for eight weeks entrances the parameters suggested for obesity. Similar mechanisms could have contributed in increased body weight, altered lipid profile, enhanced fat depositions in the present study.

When obese animals were subjected to physical exercise test for eight weeks, we found that there was significant difference (P<0.001) in body weight, lipid profile, fat deposition and blood-glucose level compared to normal control (table 1-4). Physical exercise increases the metabolic rate in the body. It enhances the digested fat to provide energy. The regular exercise was also reported to enhance the breakdown of deposited fat contributed in reduced body weight, normalize altered lipid profile, and regulate blood-glucose. The earlier research indicated that exercised animals had increased in energy expenditure through exercise training and contributed in the over body weight gain and higher free fatty acid (FFA) mobilization from other adipose tissues[16]. Our study suggests that the similar mechanism might have followed in this study too when obese animals were subjected to exercise.

The present study indicated that administration of LCD to the animals for eight weeks significantly (P<0.001) reversed the obesity parameters compared to normal control (table 1-4). The composition of LCD contains glucose, casein, Nacl, dextrin. LCD is a standard diet widely used experimentally to reduce obesity in animals. The earlier data indicated that LCD reduced obesity is due to reducing in circulating levels of glucose and lipid metabolites [17]. Our study suggests that the similar mechanism could have contributed to this study too when obese animals were substituted with LCD.

When obese animals were subjected to physical exercise and LCD test for eight weeks, we found that there was faster enhancement (P<0.001) in the reversal of obesity parameters compared to normal control (table 1-4). Physical exercise and LCD mechanism have been discussed earlier suggesting that combination of the two mechanisms could have contributed in the reduction of obesity.

The present study indicated that administration of Orlistat (200 mg/kg) to the animals for eight weeks, significantly (P<0.001) reversed the obesity parameters compared to normal control (table 1-4). Orlistat is a standard diet widely used experimentally to reduce obesity in animals. The earlier research indicated Orlistat shows pancreatic lipase inhibitor and decreased body weight pancreatic lipase inhibitor evidence by pancreatic lipase is the key enzyme for dietary fat digestion, and inhibition of the enzyme could be an effective way to alter fat absorption further data suggests that administration of Orlistat was effective in reversal of obesity parameters by the mechanism suggested in the literature [16].

Low dose of Orlistat suggests that administration of Orlistat (65 mg/kg) has also shown the significant (P<0.001) decrease in the parameters suggested for obesity (table 1-4). When obese animals were subjected to combination physical exercise and low dose Orlistat (65 mg/kg) for eight weeks, we observed that the reversal in the obesity parameters was more prominent compared to individual exercise and low dose Orlistat groups.

The results suggest that the mechanisms mentioned earlier for physical exercise, and Orlistat might have potentiated the anti-obesity action when used in combination. Similar observations were found when a combination of LCD and low dose of Orlistat was tested in obese rats, suggesting that combination in more effective than the individual treatment (table 1-4).

In addition, it was observed that triple combination of physical exercise, low dose of Orlistat (65 mg/kg) and LCD for eight weeks further suppressed the obesity parameters such as body weight, lipid profile, fat deposition and glucose metabolism in HFD rats (table 1-4). The mechanisms suggested in the earlier groups could have produced synergistic effects when used in combination. The observations indicate that exercise, low dose, Orlistat and LCD together and play a vital role in obese individual resistant to conventional anti-obesity therapies.

CONCLUSION

It can be concluded that [HFD+Orlistat (low dose)+Exercise+LCD] group was significant in reduced Body weight, Triglycerides, total cholesterol, Lipid parameters [HDL, VLDL, LDL], Blood-glucose level, organ weight [mesenteric fat and epididymal fat] and organ fat pad weight, when compared to HFD control.

Exercise shows higher free fatty acid (FFA) mobilization from adipose tissue and LCD diets exhibit little changes in circulating levels of glucose and lipid metabolites, and Orlistat shows pancreatic lipase inhibitory activity because of that reason obesity rats are reducing body weight.

The present study [HFD+Orlistat (low dose)+Exercise+LCD] group showed more potential effective than rest of all treatment groups.

CONFLICT OF INTERESTS

Declared None

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