Anti-Obesity Potential of Orlistat and Amphetamine in Rats Fed on High Fat Diet

Hanan M. Amin, Nagy S. Tawfek, Bahaa K. Abo-El Hussein and Marwa S. Abd El-Ghany

Department of Zoology, Faculty of Science, Minia University, Egypt.

ABSTRACT

Obesity, characterized by hyperleptinemia and hypogrehleninemia, has become a major health problem all over the world and is associated with an increased risk of complications including insulin resistance, hypertension, dyslipidemia, diabetes mellitus and atherosclerosis. This study was designed to assess the effectiveness of orlistat, amphetamine or the two in combination on obesity in male albino rats fed on high fat diet. Forty male rats (170 - 180 g) were divided into five groups. In the 1st group, rats received normal diet. The 2nd group fed on high fat diet. The 3rd group fed on high fat diet with intraperitoneal injection (i.p) with orlistat and the 4th group fed on high fat diet with (i.p) amphetamine, and the last group fed on high fat diet with (i.p) orlistat and amphetamine in combination. After six weeks, high fat diet group showed a significant increase in food intake, body weight, serum levels of glucose, insulin, alanine amino transaminase (ALT) and aspartate amino transaminase (AST) activities, serum leptin, apolipoprotein A-1 (Apo A1), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alpha-fetoprotein (AFP), pancreatic lipase activity, lipid peroxides represented by malondialdehyde (MDA) and total serum bilirubin and decreasing effect on superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and serum ghrelin levels. Six weeks of treatment with orlistat (12 mg/kg) or amphetamine (1.5 mg/k) produced significant decrease in body weight and food intake and significant reducing effect on TC, HDL-C and significant increase in serum ghrelin levels. On the other hand, treatment with orlistat induced significant decrease in alpha-fetoprotein, leptin, pancreatic lipase activity and total serum bilirubin and amphetamine treatment elevated AFP significantly, while, feeding on the two drugs in combination showed positive antioxidant effect by reducing lipid peroxide (MDA) significantly and anti-diabetic effect by lowering blood glucose levels significantly. We concluded that the treatment with orlistat and amphetamine had anti-obesity action and ameliorated the harmful effects of the high fat diet. Treatment with orlistat is safe and more potent than amphetamine or the two drugs in combination.

Key words: Obesity, Orlistat, Amphetamine, Pancreatic lipase, Leptin and ghrelin, lipid profile, liver functions

Introduction

It is estimated that more than 1 billion adults around the world are overweight and at least one third of this population are classified as obese. While genetic predisposition, age, and environmental factors may contribute to a person’s tendency to gain weight, it is accepted that the two primary causes of obesity are increased intake of energy-rich foods and reduced physical activity. Overweight and obesity have been important public health problems throughout the world, affecting both developed societies and developing countries (Drew et al., 2007; Pi–Sunyer et al., 2002). Also, considerable evidence indicates that obesity is directly related to increased risk of several chronic diseases and metabolic abnormalities, such as type 2 diabetes, hypertension, dyslipidemia, coronary heart disease, and certain cancers (National Task Force on the Prevention and Treatment of Obesity, 2000; Andrej et al., 2010). Glucose intolerance, hemostatic variables and increased insulin resistance are also associated with obesity (Rump et al., 2002).

The recent health crisis has spurred research in weight control, including studies in diet, exercise, surgery and pharmaceutical preparations. It has been shown that a modest reduction (5%-10%) of body weight lowers cardiovascular disease risk factor, instigates modest improvements in blood pressure and serum cholesterol and reduces the incidence of type-2 diabetes, markers of endothelial function, and inflammatory signatures (Padwel and Majumdar, 2007).

Consensus for obesity treatment is that clinical therapy should begin with lifestyle changes that focus on behavioral modification, diet, and exercise (Clinical guidelines, 1998). Some patients find that diet and exercise is not a viable option; for these patients, anti-obesity drugs can be a last resort. Anti-obesity medication or weight loss drugs are all pharmacological agents that reduce or control weight. These drugs alter one of the fundamental processes of the human body, by either altering appetite, or absorption of calories. Some prescription weight loss drugs are stimulants, which are recommended only for short-term use, and thus are of limited usefulness for extremely obese patients, who may need to reduce weight over months.

Corresponding Author: Hanan M. Amin, Department of Zoology, Faculty of Science, Minia University, Egypt
Amphetamines (alpha-methylphenethylamines) are psychostimulant drugs which stimulate the central nervous system. As well as giving the user an enhanced feeling of wakefulness and focus, it is generally agreed that the administration of amphetamine facilitates the reduction of weight. This effect of the drug was first reported in 1937, when patients receiving amphetamine for other purposes were observed to lose weight. Since that time a number of papers have appeared in which the drug has been reported to be of value in the management of obesity. However, it has not been demonstrated by controlled experiments that the drug actually causes a reduction of body weight. Moreover, using this type of weight loss medication may result in drug dependence and abuse. Also, side effects appeared with chronic use of amphetamine including irritability, personality changes, insomnia, even psychosis similar to schizophrenia. Moreover, sudden withdrawal of the drug may result in severe depression (Ioannides et al., 2005). Orlistat, a potent, specific, long-acting and reversible inhibitor of lipases, is a member of a new class of drugs available for the treatment of obesity. Orlistat plus diet has repeatedly demonstrated significantly greater weight loss, when compared to placebo plus diet. Moreover, effects of orlistat are meaningful and meet the FDA standards of efficacy for prescription weight control drugs (Mirja et al., 2004).

The safety of orlistat is supported by a comprehensive body of data including preclinical animal testing and controlled clinical trials. In other clinical trials, 8.8% of patients treated with orlistat discontinued treatment due to adverse events. Of the 40 million users of orlistat worldwide, 13 cases of severe liver damage have been reported. Other side-effect of using orlistat includes frequent, oily bowel movements (Heck et al., 2000).

The aim of this study is to evaluate and compare the ameliorating effects of orlistat, amphetamine and the two drugs in combination on high fat diet-induced obesity and obesity-related risk factors in male albino rats.

**Materials and Methods**

**Experimental Design**

Forty (40) male albino rats of sprague Dawley Strain weighing (180 - 200 g) were included in the present study. The rats were obtained from the Laboratory of Animal Colony, Minya, Egypt and were housed in well aerated cages under hygienic condition and were provided commercial rodent diet and water ad libitum for one week for adaptation. Rats were housed in temperature controlled rooms (25°C) with constant humidity and 12h/12h light/dark cycle. After the adaptation period, the rats were divided into five groups (n=8 in each group) as follows:

- **Group 1 (G1):** considered as normal control group in which rats were fed on balanced diet for six weeks.
- **Group 2 (G2):** served as positive control group in which rats were fed on high fat diet for six weeks. Also, saline solution was administered intraperitoneally to the rats of this group (1ml/kg).
- **Group 3 (G3):** represented the group that was treated with orlistat. Rats were fed on high fat diet and received (12 mg/kg) of orlistat daily dissolved in saline (1ml/kg) by intraperitoneal injection (Calderon et al., 2011) for six weeks.
- **Group 4 (G4):** represented the group that was treated with amphetamine. The animals received high fat diet and amphetamine in a dose of (1.5 mg/kg) daily dissolved in saline (1ml/kg) by intraperitoneal injection (Geigera et al., 2009) for six weeks.
- **Group 5 (G5):** this group was fed on high fat diet and received both orlistat and amphetamine in combination (i.p) for six weeks. The prepared dose was (6 mg/kg orlistat) and (0.75 mg/kg amphetamine) dissolved in saline.

**Orlistat and Amphetamine Supplementation**

Orlistat and amphetamine drugs were obtained from the pharmacy in Cairo, Egypt. Orlistat drug was marketed as a prescription under the trade name Xenical by Roche in most countries and also known as tetrahydrolipstatin, while, amphetamine is known as ("benzedrines") or alpha-methylphenethylamines.

**Composition of Balanced Diet and High Fat Diet**

Balanced diet for feeding normal control rats: 10% protein, 10% fat, 74.4 % carbohydrates, 3.5 % mineral mixture, 1 % vitamin mixture, 0.1% methionine and 1 % fiber (Pugh et al., 1999).

High fat diet for induction of obesity: 10 % protein, 30% fat, 54.4 % carbohydrates, 3.5 % mineral mixture, 1 % vitamin mixture, 0.1% methionine and 1 % fiber (Altunkaynak and B.Z., 2005).

**Body Weight Gain and Food Consumption**

Individual body weight gains were recorded before study imitation (Day 0), and weekly thereafter. Mean body weight gains were calculated for each group at each interval and for the overall testing interval. During the study, food consumptions were measured weekly per cage and mean food consumptions by individual rats were calculated.
Biochemical Analysis

At the end of the experimental period, animals were fasted overnight but allowed free access to water. Animals were also weight immediately prior to sacrifice (fasted body weight). Animals were sacrificed under anesthesia with diethyl ether, and then blood samples were immediately collected in clean and dried Wisserman tubes from the portal vein. First part of blood was collected in tubes containing potassium oxalate and sodium fluoride for the estimation of plasma glucose by O-toluidine method of Sasaki et al. (1972). Second part of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum to estimate some biochemical parameters. Serum insulin and leptin were estimated according to the methods of Wilson and Miles, (1977) and Palacio et al. (2002) respectively. (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods described by Kostener et al. (1977). Low density lipoprotein cholesterol (LDL-c) was calculated according to the method of (Frucht and Frankel, 1957). Blood superoxide dismutase (SOD) and catalase (CAT) were estimated according to the method of (Flohe and Gunzler., 1984). Lipid peroxides equivalents were determined with spectrophotometric measurement of the amount of malondialdehyde (MDA) values with thiobarbituric acid and was expressed as thiobarbituric acid reactive substances (TBARS: n mol malondialdehyde / mg protein) according to previously described method of Ohkawa et al. (1979). Glutathione (GSH) was determined according to the method of Calberg and Mannerviek, (1975). Serum total bilirubin and alpha –fetoprotein (AFP) were determined according to the method of ( Balistreri and Shaw, 1987). Determination of pancreatic lipase activity was performed according to previously described method by Tszuzki et al. (2004). Apolipoprotein A-I (APO A1) was quantified by ELISA using specific polyclonal antibodies as previously described by Navarro et al. (2005). ELISA kits for detecting rat serum ghrelin were purchased from Abscam Biochemicals, USA (Samy et al.,2013)

Statistical Analysis

Collected data were presented as mean ± SD and statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance according to Artimage and Berry (1987) and statistical significance was set at P ≤ 0.05 (Artimage et al., 1987).

Results

Gain in Body Weight and Feed Efficiency Ratio:

Table 1 shows the initial and final body weights (g) and food intake/week after feeding rats for 45 days with balanced diet, high fat diet, and high fat diet supplemented with orlistat, amphetamine or the two together. On average, the rats of G1 and G2 gained weight throughout the experimental period, when compared with the initial body weight, while significant (p<0.001) decrease was observed in G3, G4 and G5, when compared to G2. This effect was more noticed in orlistat group. Besides, there was a significant (p<0.001) increase of food intake in HFD group when compared to the normal group, while significant (p<0.001) decrease was observed in G3, G4 and G5, when compared to G2.

Table 1: The Effects of orlistat and amphetamine treatments on final body weight and food intake changes in rats fed high fat diet.

<table>
<thead>
<tr>
<th>Groups parameters</th>
<th>G1 Normal</th>
<th>G2 High fat diet (HFD)</th>
<th>G3 HFD + orlistat</th>
<th>G4 HFD +Amphetamine</th>
<th>G5 HFD+Orlistat + amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body Weight (g)</td>
<td>175 ± 1.96</td>
<td>230 ± 1.35***</td>
<td>315 ± 1.48****##</td>
<td>330 ± 2.23***###</td>
<td>310 ± 2.67****###</td>
</tr>
<tr>
<td>Final Body Weight (g)</td>
<td>193 ± 2.37</td>
<td>340 ± 1.30****</td>
<td>250 ± 1.95***###</td>
<td>295 ± 1.24***##</td>
<td>260 ± 1.77***##</td>
</tr>
<tr>
<td>Food Intake (g/week)</td>
<td>185 ± 1.09</td>
<td>270 ± 1.31***</td>
<td>230 ± 2.22***###</td>
<td>235 ± 1.79***##</td>
<td>240 ± 1.48**##</td>
</tr>
</tbody>
</table>

Significant with normal group * P<0.05 ** P<0.01 *** P<0.001
Significant with HFD group # P<0.05 ## P<0.01 ### P<0.001

Serum Lipid Profile

Table 2 shows the general serum lipid profile results. Parameters determined were triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Generally, higher values of lipid profile were seen in G2 when compared to the normal group. After treatment, there was a significant (p<0.05) reduction in TC and LDL-C levels in G3 and G4 when compared to G2, while, non-significant effect was observed after administration of the two drugs together. Little and non-significant improvement in TG levels was observed in G3, G4 and G5 when compared to G2. However, administration of orlistat, amphetamine or the two drugs in combination did not alter the levels of LDL-C significantly in G3, G4 and G5.
of serum SOD and GSH, and increase in lipid proxide (MDA) compared with the normal group. The rat groups of serum SOD, GSH and L.PEROX of the experimental rat groups G4 and G5 compared with G2. However, there was non-significant effect in serum APO A1 in G3, G4 and G5 of (CAT) in rats not significant compared with G2.

Table 2. The effect of amphetamine and orlistat treatments on lipid parameters in rats fed high fat diet.

<table>
<thead>
<tr>
<th>Groups parameters</th>
<th>G1 Normal</th>
<th>G2 High fat diet (HFD)</th>
<th>G3 HFD+ Orlistat</th>
<th>G4 HFD+Amphetamine</th>
<th>G5 HFD+Orlistat + amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (TG) mg/dl</td>
<td>92.5 ± 8.5</td>
<td>139.67 ± 0.63***</td>
<td>133.5 ± 1.79***</td>
<td>131.77 ± 4.04###</td>
<td>135.67 ± 1.22###</td>
</tr>
<tr>
<td>Total Cholesterol (TC) mg/dl</td>
<td>113.75 ± 4.26</td>
<td>223.95 ± 1.78***</td>
<td>215.42 ± 2.1#</td>
<td>219.75 ± 2.79##</td>
<td>226.3 ± 2.82***</td>
</tr>
<tr>
<td>HDL-Cholesterol (HDL-C) mg/dl</td>
<td>37.5 ± 3.22</td>
<td>83.85 ± 13.1***</td>
<td>65.6 ± 7±1.22#</td>
<td>66.05 ± 1.85#</td>
<td>69.02 ± 0.21***</td>
</tr>
<tr>
<td>LDL-Cholesterol (LDL-C) mg/dl</td>
<td>106.75 ± 2.69</td>
<td>125.97 ± 1.25***</td>
<td>126.35 ± 1.09***</td>
<td>123.35 ± 1.73***</td>
<td>129.82 ± 1.31***</td>
</tr>
</tbody>
</table>

Significant with normal group * P<0.05 ** P<0.01 *** P<0.001
Significant with HFD group # P<0.05 ## P<0.01 ### P<0.001

Serum Total Bilirubin and Pancreatic Lipase Activity

From Table 3, it is noticed that after 45 days of treatment, the total bile content of rats was different. There was non-significant increase in the serum total bilirubin in G2 compared with G1. G3 was significantly (p<0.01) lower than all the experimental groups as orlistat treatment significantly reduced the total bilirubin. This reduction was not significant after amphetamine treatment. On the other hand, administration of the two drugs increased the total bilirubin insignificantly in G5 compared to G2.

Table 3. The effect of amphetamine and orlistat treatments on Total Bilirubin, Pancreatic Lipase and Catalase (CAT) in rats fed high fat diet.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1 Normal</th>
<th>G2 High fat diet (HFD)</th>
<th>G3 HFD+Orlistat</th>
<th>G4 HFD+Amphetamine</th>
<th>G5 HFD+Orlistat + amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin mg/dL</td>
<td>1.07 ± 4.78</td>
<td>1.37 ± 8.53</td>
<td>0.85 ± 0.13#</td>
<td>1.3 ± 0.18</td>
<td>1.45 ± 0.2*</td>
</tr>
<tr>
<td>Pancreatic Lipase U/L</td>
<td>15.00 ± 0.91</td>
<td>25.60 ± 0.32***</td>
<td>17.32 ± 0.32##</td>
<td>26.60 ± 1.94###</td>
<td>28.25 ± 0.6***</td>
</tr>
<tr>
<td>Catalase U/L</td>
<td>3.42 ± 0.36</td>
<td>1.65 ± 6.45**</td>
<td>1.6 ± 4**</td>
<td>1.6 ± 0.26##</td>
<td>1.4 ± 0.4*</td>
</tr>
</tbody>
</table>

Significant with normal group * P<0.05 ** P<0.01 *** P<0.001
Significant with HFD group # P<0.05 ## P<0.01 ### P<0.001

Serum ALT, AST, AFP and APO A-1 of the experimental rat groups

Table 4 illustrated that HFD in G2 increased serum AST & ALT enzymes significantly (P<0.001 and p<0.05), as compared with G1. However, there was no significant improvement in Serum AST&T ALT enzymes in all treated groups compared with G2. Table 4 reveals that feeding with HFD resulted in significant increase in alpha fetoprotein and apolipoprotein A1 in rats as compared to G1 (p<0.05 and P<0.001). Treatment with orlistat (G3) decreased AFP levels significantly, On the other hand, amphetamine treatment increased serum AFP significantly compared with G2. However, there was no-significant effect in serum APO A1 in G3, G4 and G5 compared with G2.

Table 4: Serum ALT, AST, AFP and APO A-1 of the experimental rat groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1 Normal</th>
<th>G2 High fat diet (HFD)</th>
<th>G3 HFD+Orlistat</th>
<th>G4 HFD+Amphetamine</th>
<th>G5 HFD+Orlistat + amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U /ml)</td>
<td>47.5 ± 1.19</td>
<td>85.2 ± 1.64***</td>
<td>82.8 ± 5.69***</td>
<td>79.4 ± 5.89**</td>
<td>78.92 ± 5.0**</td>
</tr>
<tr>
<td>AST (U /ml)</td>
<td>45.5 ± 2.1</td>
<td>57.23 ± 0.87*</td>
<td>55.52 ± 5.4*</td>
<td>53.57 ± 3.41*</td>
<td>52.35 ± 4.9*</td>
</tr>
<tr>
<td>AFP Ng/ml</td>
<td>18.25 ± 0.85</td>
<td>23.3 ± 1.58*</td>
<td>15.62 ± 1.12##</td>
<td>35.02 ± 0.64##</td>
<td>21.95 ± 1.4</td>
</tr>
<tr>
<td>APO A-1 Ng/ml</td>
<td>58.57 ± 2.42</td>
<td>88.4 ± 2.61***</td>
<td>81.9 ± 6.11**</td>
<td>79.95 ± 16.86##</td>
<td>78.34 ± 2.73##</td>
</tr>
</tbody>
</table>

Significant with normal group * P<0.05 ** P<0.01 *** P<0.001
Significant with HFD group # P<0.05 ## P<0.01 ### P<0.001

Serum SOD, GSH and L.PEROX of the experimental rat groups

Data presented in table (5) showed that HFD had significant (p<0.01 and p<0.001) reduction in the values of serum SOD and GSH, and increase in lipid proxide (MDA) compared with the normal group. The rat groups
that were treated with orlistat and amphetamine showed non-significant improvement in the values of serum SOD, GSH and MDA compared with G2, while, administration of amphetamine and orlistat together showed significant decreasing effect on lipid peroxides (MDA).

**Table 5: Serum SOD, GSH and L.PEROX of the experimental rat groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1 Normal</th>
<th>G2 High fat diet (HFD)</th>
<th>G3 HFD + Orlistat</th>
<th>G4 HFD + amphetamine</th>
<th>G5 HFD + Orlistat + amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD U/mL</td>
<td>1.35 ± 6.45</td>
<td>0.78 ± 4.27**</td>
<td>0.9 ± 4.08*</td>
<td>0.85 ± 0.23*</td>
<td>0.6 ± 0.4***</td>
</tr>
<tr>
<td>GSH Mgl</td>
<td>5.15 ± 0.43</td>
<td>1.21 ± 4.23**</td>
<td>1.3 ± 7.07***</td>
<td>1.27 ± 0.3***</td>
<td>1.07 ± 0.9***</td>
</tr>
<tr>
<td>L.PEROX (MDA)</td>
<td>0.2 ± 4.08</td>
<td>2.25 ± 6.45**</td>
<td>2.15 ± 5***</td>
<td>2.15 ± 0.27***</td>
<td>1.9 ± 0.7***</td>
</tr>
</tbody>
</table>

Significant with normal group * P<0.05 ** P<0.01 *** P<0.001
Significant with HFD group # P<0.05 ## P<0.01 ### P<0.001

**Blood Glucose, Serum Insulin**

Table (6) reveals that feeding with HFD resulted in hyperglycemia and hyperinsulinemia in G2 compared to G1. Administration of orlistat or amphetamine in G3 and G4 showed non-significant improvements in serum glucose and insulin as compared to G2. Conversely, administration of the two drugs in G5 resulted in significance (p<0.05) reduction in glucose level and the best improvement in insulin level compared with G2.

**Serum Leptin and Ghrelin**

Table (6) showed that serum leptin increased significantly (p>0.001) in response to HFD in G2 as compared with G1. Administration of orlistat alone resulted in the best ameliorating effect on serum leptin levels represented in significant (p<0.01) decreasing effect compared with G2. The present data showed that the serum ghrelin decreased significantly (p<0.05) in response to HFD as compared with G1. Additionally, administration of orlistat, amphetamine or the two together in G3, G4 and G5 significantly (p<0.001, p<0.01 and p<0.05) increased the serum ghrelin levels as compared with G2. The best effect is noticed in the orlistat group.

**Table 6. Serum levels of leptin, ghrelin, glucose and insulin in the experimental groups.**

<table>
<thead>
<tr>
<th>Groups Parameter</th>
<th>G1 Normal</th>
<th>G2 High fat diet (HFD)</th>
<th>G3 HFD + Orlistat</th>
<th>G4 HFD + amphetamine</th>
<th>G5 HFD + Orlistat + amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>81.25 ± 4.26</td>
<td>119.25 ± 3.95*</td>
<td>115.4 ± 5.95*</td>
<td>119.47 ± 17.18*</td>
<td>91.56 ± 2.39#</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.3 ± .51</td>
<td>5.82 ± 26***</td>
<td>5.75 ± 29***</td>
<td>5.72 ± 99***</td>
<td>4.75 ± 35##</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.2 ± .057</td>
<td>12.81 ± .057***</td>
<td>5.68 ± 2.03##</td>
<td>10 ± .82##</td>
<td>11.47 ± 1.72##</td>
</tr>
<tr>
<td>Ghrelin (ng/ml)</td>
<td>1.3 ± .057</td>
<td>.46 ± .005*</td>
<td>7.14 ± .97##***</td>
<td>3.53 ± 7.1##</td>
<td>3.89 ± 1.32####</td>
</tr>
</tbody>
</table>

Significant with normal group * P<0.05 ** P<0.01 *** P<0.001 Significant with HFD group # P<0.05 ## P<0.01 ### P<0.001

**Discussion**

Obesity is characterized by increased adipose tissue mass that results from both increased fat cell number and increased fat cell size. Adipose tissue is a dynamic organ that plays an important role in energy balance and changes in mass according to the metabolic requirements of the organism (National Institutes of Health, 1998). Because obesity is caused by a build-up of fat in the body due to, for example, the over-consumption of high fat food, modern therapeutic approaches are mostly focused on blocking or stimulating various biomolecules and enzymes involved in fat metabolism and an increase in mass of adipose tissue, confers a higher risk for metabolic diseases such as type 2 diabetes, cardiovascular disease, and an increased incidence of morbidity (Wickelgren, 1998).

Our results demonstrated that the rats in the high fat diet (HFD) group (G2) gained weight throughout the experimental period, when compared to the initial body weights and the rats in the normal group (G1). This may be due to HFD causing hyperphagia which is similar to human cafeteria diet. Both palatability and energy density contribute to fat hyperphagia and reduced satiation signaling accompanying HFD consumption which can contribute to overconsumption and often lead to obesity (Sjostrom et al., 1998). On the other hand significant decrease was observed in body weight after treatment with orlistat, amphetamine or the two together. Besides, there was a significant increase of food intake in HFD group that was improved significantly in all treated groups. It is generally agreed that the administration of amphetamine facilitates the reduction of body weight by suppressing appetite (Ioannides, et al., 2005). This effect was proved to orlistat in our study that showed that the effect of orlistat alone on weight loss was more potent than the two other treated groups. pancreatic lipase is the enzyme that break down triglycerides in the intestine. When lipase activity is blocked,
triglycerides from the diet are not hydrolyzed into absorbable free fatty acids, and are excreted undigested instead (David and S.W., 2003). In our results, the levels of pancreatic lipase increased significantly in response to HFD and only administration of orlistat had positive decreasing effect on this enzyme. It is known that orlistat is the saturated derivative of lipstatin, a potent natural inhibitor of pancreatic lipases isolated from the bacterium Streptomyces toxytricini. However, due to simplicity and stability, orlistat rather than lipstatin was developed into an anti-obesity drug. Because this drug can reduce the absorption of dietary fat by up to 30%, it produces weight loss comparable to or greater than that obtained by placing an individual on a fat-restricted diet (David and S.W., 2003). Amphetamine treatment did not induce any significant effect on pancreatic lipase level as observed in G4. Previous studies found that some natural sources and aqueous extracts of some medicinal herbs can improve body weights and did not possess any lipase inhibition. This means that amphetamine may able to reduce lipase activity by reducing appetite, without alterations in pancreatic lipase level (Ioannides et al., 2005).

Our study showed that higher values of serum lipid profile were observed in HFD group when compared to the normal group. A high level of cholesterol is one of the most common problems among overweight or obese people, and this can over a period of time, cause several other complications, including coronary heart diseases and heart attack (Wickelgren, 1998). There was a significant decreasing effect on TC, and HDL-C levels in orlistat and amphetamine groups when compared to HFD group, while, there was no observed effect for these drugs on LDL-C. On the other hand, little and non-significant improvement in TG levels were observed in G3, G4 and G5 when compared to G2. These results indicate that treating obese rats with orlistat and amphetamine had positive ameliorating effect on the lipid fractions and administration of each of them alone is better than the two in combination. It was reported that, improvement in concentrations of cholesterol resulted from therapy with orlistat is a result of its effect on the body's ability to absorb dietary fats; orlistat is known to be associated with an increased incidence of gastrointestinal events in its users, in addition to, its positive decreasing effect on pancreatic lipase activity (Pi et al., 1996). It alters energy balance by reducing the absorption of triglyceride and cholesterol from the gastrointestinal tract (Mittendorfer et al., 2001). Reduction of the enzymatic activity is mediated through the covalent binding of orlistat to the serine residue of the lipase active site (Asler et al., 2007, Guerciolini and R., 1997). Thus, an inhibitor of digestive lipase that helps to limit intestinal fat absorption could be proved as useful medication for the treatment of hyperlipidemia and holds great promise as an anti-obesity agent.

Apolipoprotein A-I (APO A1) is the major protein component of HDL-c in plasma that promotes cholesterol efflux from tissues to the liver for excretion, and it is a cofactor for lecithin cholesterol acyltransferase (LCAT) which is responsible for the formation of most plasma cholesteryl esters. It is a simple clinical indicator and its level is closely related with coronary heart diseases in overweight and obese patients (Rossner et al., 2000). Treatment with orlistat or amphetamine did not have significant change in the high levels of APO A1 induced by HFD in the present study.

Serum AST and ALT levels are clinically and toxicologically important indicators and increase as a result of tissue damage caused by toxicants or disease conditions. Their significantly elevated levels after administration of HFD were not affected significantly by treatment with orlistat or amphetamine indicating that these drugs cannot dramatically suppress the development of HFD-induced fatty liver (Han et al., 2001). Orlistat is generally well tolerated and was approved by the Food and Drug Administration in 1998 and its safety has been supported by a comprehensive body of data (Mirja et al., 2004). However, severe liver injury and hepatic failure has been reported in controlled clinical trials in which patients treated with orlistat discontinued treatment due to adverse events (Heck et al., 2000). Alpha-fetoprotein (AFP) is a type of protein produced in the developing fetus and found in adults. If an individual has high levels of alpha-fetoprotein in the blood, it may be a sign of liver damage, failure or even liver cancer. In the present study, orlistat significantly decreased the high levels of AFP induced by HFD. These results support the safety of orlistat and indicate that it may have ameliorating effect on liver damage or function. Conversely, amphetamine treatment increased serum AFP significantly indicating that treatment with that drug is not safe and severe liver injury and hepatic dysfunction may be associated with treatment with this medication (Sharma et al., 2005).

Obesity is a disorder of energy balance and is associated with high blood glucose levels, hyperinsulinemia and insulin resistance that contribute to vascular dysfunction and type 2 diabetes (Wickelgren, 1998). These findings are in agreement with our results that demonstrated that HFD resulted in hyperglycemia and hyperinsulinemia and that orlistat and amphetamine showed significant hypoglycemic action, only, when administered together in (G5). In this respect, previous study reported that, the addition of orlistat to a conventional weight loss regimen significantly improved oral glucose tolerance and diminished the rate of progression to the development of impaired glucose tolerance and type 2 diabetes (Steven et al., 2000). However, no previous studies have explained the mechanism by which amphetamine can exert its effect on glucose level. However, it may have insulin-like action and exerts a blood glucose suppressing effect by improving insulin sensitivity signaling and synthesis.

Bilirubin is a waste product made from old blood cells. Tests for bilirubin levels help to determine if the liver is functioning appropriately (Hafkamp et al., 2003). Results of the present study showed increase in the
total content of bilirubin in HFD group compared to the normal group. On the other hand, only orlistat group had a significant lowering action in the total content of bilirubin comparing with G2. It was hypothesized that orlistat treatment decreases plasma bilirubin concentration in rats by increasing turnover and fecal excretion of bilirubin (Yesilbursa et al., 2005).

Studies had suggested that obesity is associated with increased oxidative stress and may be a state of chronic oxidative stress. In addition, Oxidative stress may be the mechanism underlying the development of co-morbidities in obesity. On the other hand, lipid peroxidation is associated with several indices of adiposity and a low systemic antioxidant defence (i.e. antioxidant enzymes, tissue dietary antioxidants, glutathione). The high level of oxidative stress associated with the increased lipid peroxidation may be one of the reasons why those who are overweight are at greater risk for developing heart disease (Kayan et al., 2005). From our results, we can conclude that there is a significant relationship between fatty diet intake and increased oxidative stress monitored by increased levels of lipid peroxide (MDA) whereas GSH, CAT and SOD levels decreased significantly. While our study indicated that treatment of HFD rats with orlistat or amphetamine did not produce significant decrease in lipid peroxide (MDA) or increase in GSH, SOD or CAT, significant decrease in serum MDA was realized when the two drugs were administrated in combination, indicating limited antioxidant effect for both amphetamine and orlistat. These results are not in parallel to other studies that indicated that treatment of HFD rats with Orlistat produced significant increase in serum CAT, GSH and SOD and a high significant decrease in MDA as compared to the obese, untreated rats (Meiste, 1988).

Leptin and ghrelin are two hormones that have been recognized to have a major influence on energy balance. Leptin is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss (Haslam et al., 2005). Ghrelin, on the other hand, is a fast-acting hormone, seemingly playing a role in meal initiation. It is a 28-amino acid orexigenic peptide produced mainly by the stomach that is involved in both the long-term and short-term regulation of postprandial satiety. Impairments in ghrelin secretion may in concert with other factors play an important role in the development of both obesity and anorexia nervosa (Hamann et al., 1996). Leptin is produced peripherally, and control appetite through their actions on the central nervous system, thus, a deficiency in leptin signaling, either via leptin deficiency or leptin resistance, leads to overfeeding and may account for some genetic and acquired forms of obesity. Leptin resistance, as it's known, results in "unnecessarily high food intake," and low leptin levels are a precursor to obesity (Faggioni et al., 2000). However, the manner in which both the leptin and ghrelin systems contribute to the development or maintenance of obesity is as yet not clear.

In obese subjects the circulating level of the orexigenic hormone ghrelin is decreased. These findings are in agreement with various studies (Hamann et al., 1996) and in parallel to our results that showed significant reduction in ghrelin levels and significant increase in leptin levels in response to HFD compared with the control non obese rats. Only administration of orlistat in G3 resulted in significant ameliorating effect on serum leptin. Serum leptin concentration is directly related to obesity severity, as an increase of fat mass is associated with an increase of leptin which makes leptin an indicator of the total fat mass (Lopez et al., 2005). The decreased levels of leptin in the present study in response to orlistat highlight its role in reducing total fat mass and improving obesity. Moreover, leptin is produced by adipose tissue to signal fat storage reserves in the body, and mediates long-term appetite controls (Faggioni et al., 2000).

Administration of orlistat, amphetamine or the two together in G3, G4 and G5 significantly increased the serum ghrelin level as compared with G2. The best effect is noticed in the orlistat group. These results indicate positive effects for both orlistat and amphetamine on weight loss. However, obesity is characterized by hyperleptinemia and hypogghrelinemia and as orlistat could improve and normalize the levels of both leptin and ghrelin, it could be better anti-obesity agent than amphetamine that affects the level of ghrelin only.

**Conclusion**

It could be conclude that both orlistat and amphetamine can modify high fat diet –induced obesity. Orlistat exerts its effect by inhibiting pancreatic lipase levels and amphetamine may reduce weight by suppressing appetite. The two drugs have potential lipid lowering properties and limited antioxidant action and regulatory role in blood glucose and insulin levels. Treatment with orlistat alone is more potent than treatment with amphetamine or the two drugs in combination in reducing weight and ameliorating the weight-related medical complications. Moreover, treatment with orlistat is safe, while, administration of amphetamine is associated with severe side effects including liver damage.

**References**


