The Effect of Orlistat and Metformin Treatment on Body Weight, Liver Steatsis, Leptin and Insulin Sensitivity in Obese Rats Fed High Fat Diet

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Abstract: The anti-obesity drugs are indicated for obesity management when used in conjunction with reduced calorie diet, some researches hesitate to use the medical treatment for NAFLD. The aim of the research is to study the short term effect of orlistat and metformin on body weight and liver histology in obese rats feeding high fat diet. The obesity was induced by feeding wistar female rats with high fat diet (HF 45%) for 12 weeks with a control group low fat diet (LF 10%), the obese rats divided to three subgroups: the first group treated with orlista, the second group treated with metformin, the third group without treatment (placebo) for 4 weeks with feeding high fat diet. The food intake and body weight were recorded. At the end of experimental period, the animals were sacrificed, blood samples were collected for biochemical and hormonal measurements with liver histological study. Treatment obese rats for 4 weeks with orlistat and metformin reduced significantly ($p<0.05$) food and energy intake (65.473±0.767 gm/week, 68.11±0.363gm/week; 309.69±3.632 kcal/week, 321.04±3.602 kcal/week), body weight (320.54±2.291 gm, 319.347±2.518 gm), BMI (0.655±0.009, 0.653±0.013), LOI (0.309±0.001, 0.309±0.002) and AI (8.43±0.560, 8.26±0.852), in addition to modulate plasma Leptin and Insulin concentrations (3.50±0.437, 3.02±0.682ng/ml ; 0.770±0.094, 0.651±0.129 ng/ml) and improve Insulin sensitivity by reducing fasting plasma glucose and HOMI (12.62±0.321mmol/L, 12.25±0.500mmol/L; 9.17±0.867, 7.75±0.596). The two drugs had favorable effects on fasting plasma lipids and lipoprotein concentrations. Liver steatosis was decreased in both drugs and decreased levels of liver ALT, AST(131.375±0.929, 167.751±0.350; 115.600 ±0.620, 157.497 ±0.306 IU/L). Treated obese rats with orlistat or metformin can correct obesity, Leptin and Insulin resistance in high fat fed obese rats and have anti atherogenic properties in addition to improve liver function and histological response in NAFLD.
[Sanaa Jameel Thamer. **The Effect of Orlistat and Metformin Treatment on Body Weight, Liver Steatsis, Leptin and Insulin Sensitivity in Obese Rats Fed High Fat Diet.** *J Am Sci* 2014;10(4):107-114]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 14

Key words: anti obesity drugs, liver steatosis, Leptin and Insulin sensitivity.

1.Introduction

Obesity is the most public health problems distributed in the world that associated with type 2 diabetes, cardiovascular diseases, dyslipidaemia and non alcoholic fatty liver disease NAFLD (Centers for Disease Control and Prevention, 2009), it resulted from disordered in energy balance that increasing body fat mass which caused metabolic syndrome (Kopelman, 2000). Adipose tissue conceder as endocrine gland that secret hormone Leptin controlling energy homeostasis by regulation food intake and energy balance (Considine *et al.*,1996). Human obesity characterized by hyperlipidaemia, elevated levels of plasma Leptin and Insulin concentrations that reflect Leptin and Insulin resistance (Zhang *et al.*, 1994; De Ferranti & Mozaffarian, 2008). Because the relation between obesity and type 2 diabetes with NAFLD, many adequate treatments were suggested to reduced metabolic syndrome included change life style, diet and exercises (Freemark, 2007), other strategy based on hypoglycemic and lipid lowering effect using anti-obesity drugs that developed to reduce body weight in overweight patients, orlistat belong to this group that reduce diatery fat absorption (Hollander *et al.* ,1998; Karhunen *et al.*, 2000) and improve Insulin sensitivity (Karhunen *et al.*, 2000), while the anti-hyperglycemic drug metformin used to treat type 2 diabetes by acting as hypoInsulinemia, improve hepatic Insulin resistance (Zuhri-Yafi *et al.*, 2002) and promote weight loss (Freemark & Bursey, 2001). Some researches referred to the positive role of orlista or metformin in body weight management that combined with appropriate diet control (Davidson *et al.*,1999; Hauptman *et al.*,2000) but in our research we conduct on the role of the two drugs to correct obesity in animals in combination with fat rich diet. A number of clinical trial have used metformin to treat NAFLD (Lam & Younossi, 2010), however some researches referred to improve metabolic variable in obese patients (Mazza *et al.*, 2012) while others referred to ineffective treatment on liver steatosis (Rakoski *et al.*, 2010) also some trials reported several limits included incomplete histological outcomes in addition to the effect of orlistat and metformin on liver histology remain unclear, therefore the aim is study the short term effect of anti-obesity drugs on body weight, Leptin

and Insulin sensitivity and the association of these two drugs with the degree of liver steatosis in obese rats fed high fat diet.

2.Materials and methods

Diet: Diet induced obesity in rodents (HF 45% fat) and its control (LF 10% fat) was formulated according to the Research Diet INC. (2004). The composition of the experimental diet shows in table 1.

Table 1: composition of the experimental diets in the study.

Ingredients	Control (10 % fat)	HF (45 % fat)
Casein	200	200
L- cysteine	3	3
Cornstarch	315	72.8
Sucrose	385	272.8
Cellulose powder	50	50
Soy bean oil	25	25
Beef tallow	20	177.5
Mineral mixture	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5
Potassium citrate	16.5	16.5
Vitamin mixture	10	10
Choline bitartarate	2	2
Total weight gm Total Kcal Total Kcal/ gm	1055 4057 3.85	858.1 4057 4.73

Animals:

Female Wister albino rats (6 weeks aged and 97± 10 gm weight) were acclimatizing on low fat diet for one week before introducing to the experimental diets, the animals either feeding on low fat diet (control diet LF: 10% energy from tallow) n=12 or on high fat diet (HF 45% energy from tallow) n=24 for 12 weeks (table 1). All animals were kept in constant room temperature (25-30 ^{o}C) and 12:12 h light: dark cycle with free access to food and water. The obese rats from high fat diet group were divided into the following subgroups (n= 8). The first subgroup was treated with orlistat (120 mg/kg twice daily), the second was treated with metformin (500 mg/kg twice daily), by dissolved the drug in distilled water and administered orally, while the third subgroup treated with distilled water (placebo) for 4 weeks, the animals feeding with high fat diet. Food consumed and energy intake were recorded daily. At the end of experimental period, animals (n=3) from each subgroup were sacrificed, blood samples and tissues were collected and stored at -70 ^{o}C . Adiposity index was calculated according to Tayler & Phillips (1996): AI = [Weight of fat pads (gm) ÷ body weight (gm)] × 100. Obesity was determined by the Lee index at the end of each dietary experimental group according to Bernardis (1970). LOI= $\sqrt[3]{}$body weight (gm)÷ naso-anal length (cm). Body mass index was calculated according to Novelli *et al*.(2007), BMI: Body weight (gm) ÷ Length ^{2} (cm).

Biochemical parameters:

Plasma glucose, total cholesterol (T-ch), triglycerides (TG) concentrations were measured by enzymatic method using diagnostic kit from Randox (UK) and Biolabo companies (France). Low density lipoprotein (LDL) was calculated according to the formula of Friedewal *et al*. (1972): LDL cholesterol= T.ch - HDL - (TG/5), very low density lipoprotein (VLDL) and Phospholipids were measured according to the formulae of Tietz (1976): VLDL= TG/5, Phospholipids = 68 + (T-ch × 0.89). Liver alanine transamianse (ALT) and aspartate transaminase (AST) were measured by enzymatic method using diagnostic kit from Biolabo companies (France).

Hormonal measurements:

Plasma rat Leptin and Insulin concentrations (ng/ml) were measured using Rat Elisa kit from CRYSTAL CHEM INC (for Leptin cat no. 90040 USA, for Insulin cat no. 90010 USA).

Insulin resistance:

The homeostatic index of Insulin resistance (HOMA-IR) was calculated according to the equation developed by Matthews *et al*. (1985):

HOMA-IR = [Glucose (mmol/L) × Insulin (pmol/L)] ÷ 155.

To converting Insulin from ng/ml to pmol/L: multiplying by 150 (Lee *et al*.,2005).

Histological Study:

Liver histological sections were prepared according to Luna (1968) and stained with haematoxylin and eosin dyes.

Statistical analysis:

Data were analyzed by one- way or two- way ANOVA using a general liner model procedure using SPSS version 15 statistic program. Comparisons between means were made using least significant differences (LSD). Differences were considered to be significant at $p<0.05$. Data are presented as means ± standard deviation.

3.Results:

The high fat fed obese rats:

The food consumed and energy intake were increased significantly ($p<0.05$) in rats fed high fat diet compared to rats fed on standard (low fat diet) this influence significant increasing in body weight that reflected high values in BMI, AI and LOI in HF group compared to control group. Fasting plasma lipids, lipoprotein, Leptin and Insulin concentrations

were significantly higher in high fat fed obese rats (table 2).

Table 2: Food consumed, body weight, biochemical and hormonal parameters in control and high fat fed rats. Means ±S.D.($p<0.05$).

	Control fed rats	High fat fed rats
Food consumed gm /week	72.52 ± 1.889b	78.82 ± 0.928a
Energy intake kcal/week	279.2 ± 4.274b	373.0 ± 3.141a
Body weight gm	243.75 ± 1.574b	338.52 ± 0.815a
BMI	0.503 ± 0.003b	0.699 ± 0.002a
AI	5.680 ± 0.054b	9.300 ± 0.005a
LOI	0.283 ± 0.005b	0.316 ± 0.004a
T-ch mmol/L	1.815 ± 0.022b	3.733 ± 0.057a
TG mmol/L	0.848 ±0.005b	1.480 ± 0.004a
HDL mmol/L	0.768 ± 0.001a	0.589 ± 0.005b
LDL mmol/L	0.877 ± 0.022b	2.848 ± 0.064a
VLDL mmol/L	0.169 ± 0.011b	0.295 ± 0.005a
Leptin ng/ml	1.455 ± 0.039b	5.401 ± 0.051a
Insulin ng/ml	0.626 ± 0.004b	0.992 ± 0.004a
Gluc. mmol/L	11.890 ± 0.029b	14.347 ± 0.054a
HOMI	7.202 ±0.041b	13.777 ± 0.109a

The effect of orlistat and metformin treatment:

Food consumed and energy intake:

The obese rats treated with orlistant or metformin were significantly ($p<0.05$) decreased their food and caloric intake (65.473±0.767, 68.11± 0.363gm; 309.69±3.632, 321.04±3.602 kcal/week) during the 4 weeks of treatment compared to obese rats without treatment (84.67 ± 1.527 gm; 400.473 ± 2.225 kcal/week). Orlistat was more effective with significant differences than metformin (figures 1 and 2).

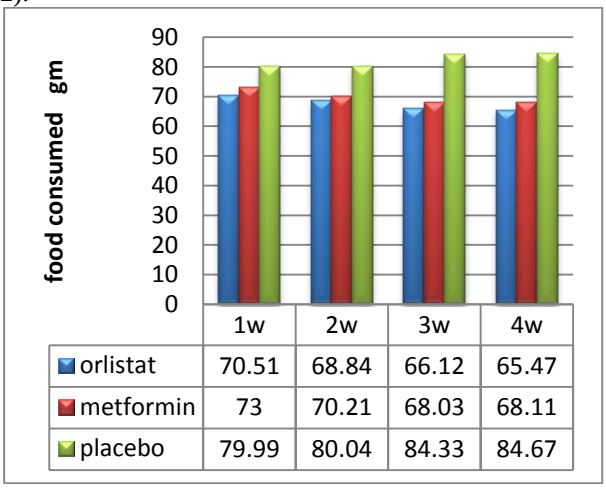


Figure 1: Effect of orlistat and metformin administration on food consumed in high fat fed obese rats. Means ($p<0.05$).

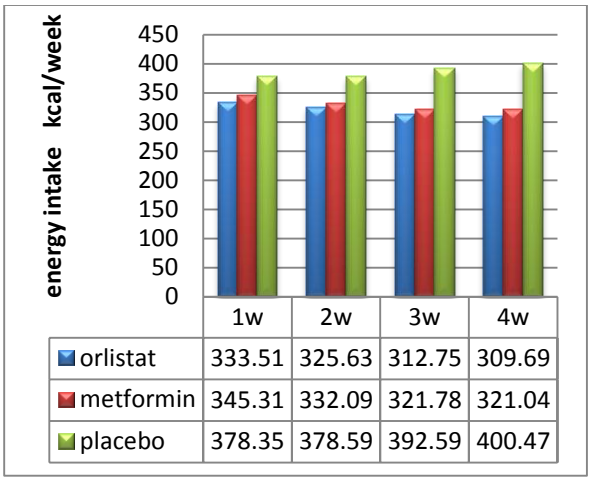


Figure 2: Effect of orlistat and metformin administration on energy intake in high fat fed obese rats. Means ($p<0.05$).

Body weight:

The body weight was significantly ($p<0.05$) reduced in obese rats after administration of anti-obesity drugs especially in the third and fourth week of treatment without significant differences between the two drugs (320.54 ±2.291gm, 319.347±2.518 gm). This decreasing in obesity characterized the treated rats by reducing body weight parameters BMI, LOI and reduced retroperitoneal adipose tissue AI (0.655 ± 0.009, 0.653 ± 0.013; 0.309 ± 0.001, 0.309 ± 0.002 and 8.43 ± 0.560, 8.26 ± 0.852 respectively) (figure 3, table 3) compared to high parameters in placebo group.

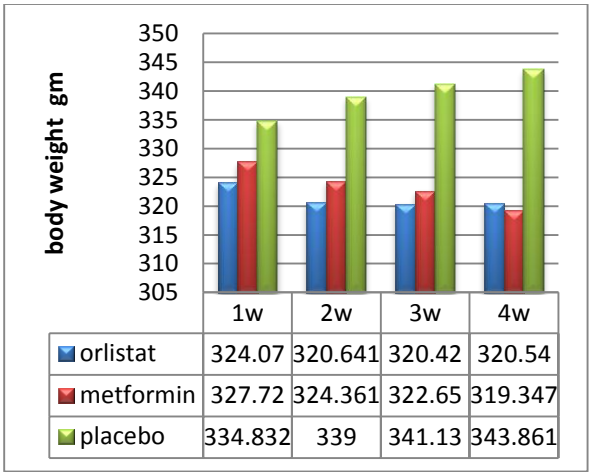


Figure 3: Effect of orlistat and metformin administration on body weight in high fat fed obese rats. Means ($p<0.05$).

Table 3: Effect of orlistat and metformin administration on weight parameters in high fat fed obese rats. Means ±S.D (p<0.05).

Treatment	BMI	LOI	AI
Orlistat	0.655 ± 0.009b	0.309 ± 0.001b	8.43 ± 0.560b
Metformin	0.653 ± 0.013b	0.309 ± 0.002b	8.26 ± 0.852b
Placebo	0.699 ± 0.011a	0.321 ± 0.004a	9.62 ± 0.035a

Biochemical parameters:

Fasting plasma glucose, lipoprotein concentrations included total cholesterol (T.ch), triglycerides (TG), low and very low density lipoproteins (LDL, VLDL) were significantly (p<0.05) decreased after treatment the obese rats with orlistat or metformin drugs for 4 weeks, while the high density lipoprotein concentration (HDL) was significantly (p<0.05) enhanced after the treatments (table 4).

Table 4: Effect of orlistat and metformin administration on biochemical parameters in high fat fed obese rats. Means ± S.D. (p<0.05).

	Orlistat	Metformin	placebo
Gluc. mmol/L	12.62 ±0.321b	12.25 ±0.500b	14.79 ±0.005a
T.ch mmol/L	3.070 ±0.300c	3.141 ±0.233b	4.384 ±0.110a
TG mmol/L	0.797 ±0.031b	0.832 ±0.079b	1.495 ±0.495a
HDL mmol/L	0.733 ±0.057a	0.688 ±0.059a	0.561 ±0.003b
LDL mmol/L	2.178 ±0.352b	2.287 ±0.273b	3.524 ±0.106a
VLDL mmol/L	0.159 ±0.006b	0.166 ±0.015b	0.298 ±0.001a

Plasma levels of Leptin, Insulin concentration and HOMI:

Fasting plasma levels of Leptin and Insulin concentration were significantly (p<0.05) decreased after treatment with anti-obesity drugs compared to the high level in obese rats in placebo. The reduction in glucose and Insulin concentrations were effective to improve Insulin sensitivity by reducing HOMI in the treated animals (table 5).

Table 5: Effect of orlistat and metformin administration on hormonal measurements in high fat fed obese rats. Means ± S.D. (p<0.05).

Treatment	Leptin ng/ml	Insulin ng/ml	HOMI
Orlistat	3.50 ±0.437b	0.770 ±0.094b	9.17 ±0.867b
Metformin	3.02 ±0.682b	0.651 ±0.129b	7.75 ±0.596b
Placebo	7.15 ±0.326a	0.994 ±0.015a	14.23 ±0.230a

Liver functions test:

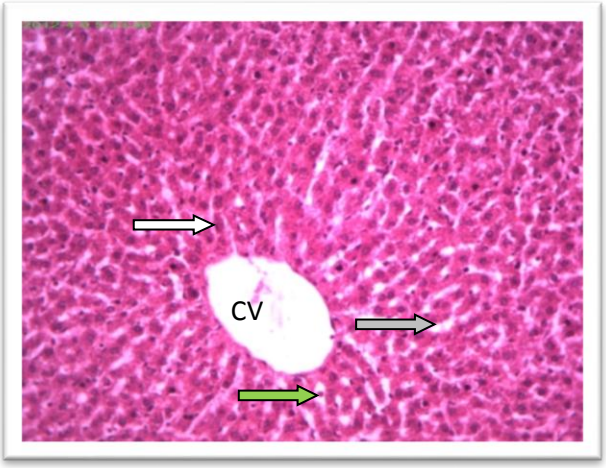
Liver enzymes ALT and AST were significantly (p<0.05) decreased in obese rats administrated with orlistat or metformin for 4 weeks (table 6) while the animals in placebo group showed high levels in both enzymes.

Table 6: Effect of orlistat and metformin administration on liver enzymes in high fat fed obese rats. Means ± S.D. (p<0.05).

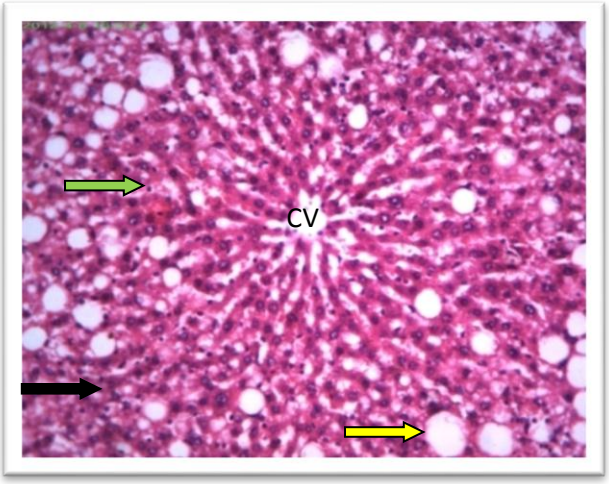
Treatment	ALT IU/L	AST IU/L
Orlistat	131.375±0.929b	167.751±0.350b
Metformin	115.600 ±0.620c	157.497 ±0.306c
Placebo	156.621 ±1.200a	193.156 ± 0.754a

Liver tissues:

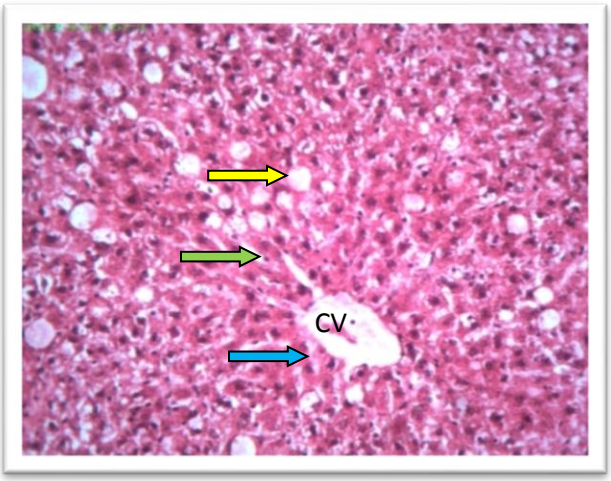
Haematoxylin and eosin liver section in control group showed classic formed of liver hepatocytes that arranged radial around the central vein with normal sinusoids (picture 1). The liver section of obese rats fed high fat diet characterized by peripheral steatosis (accumulation of intra-cytoplasmic fat droplets) in parenchyma cells with inflammation and increased number of kupffer cells (picture 2). Treated with orlistat caused histological response in hepatic steatosis. Haematoxylin and eosin section showed mild reduction in fat storage cells and hepatocytes regenerated special around the central vein also decreased number of kupffer cells (picture 3). The vascular steatosis was diminished in most part of liver tissue after treatment with metformin, the hepatocytes regenerated and had radial arrangement around the central vein with relatively normal kupffer cells and sinusoids, no signs of inflammation and vascular dilation were observed (picture 4).



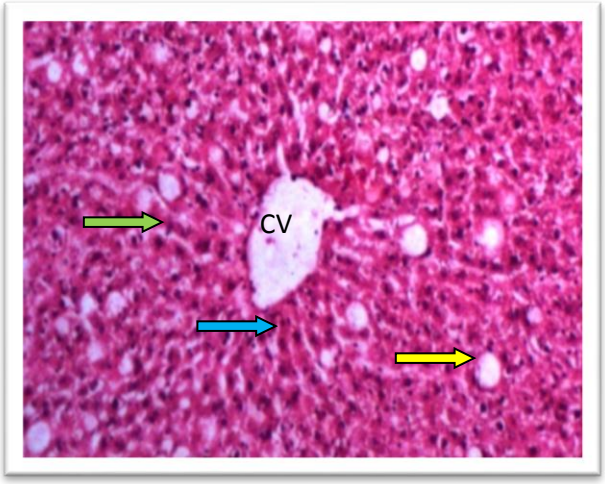
Picture 1: H and E liver section of rat fed low fat diet (control group LF: 10% tallow) for 12 weeks. CV(central vein), hepatocyte (white arrow), normal sinusoid (gray arrow), kupffer cells (green arrow). Magnification power 200 x.



Picture 2: H and E liver section of rat fed high fat diet (HF 45% tallow) for 12 weeks showed hepatic steatosis. CV(central vein), residual lipid vacuole (yellow arrow), kupffer cells (green arrow), degenerated hepatocytes (black arrow). Magnification power 200 x.



Picture 3: H and E liver section of rat treated with orlistat drug for 4 weeks showed mild hepatic steatosis. CV(central vein), residual lipid vacuole (yellow arrow), kupffer cells (green arrow), regenerated hepatocytes (blue arrow). Magnification power 200 x.



Picture 4: H and E liver section of rat treated with metformin drug for 12 weeks showed recovery from steatosis. CV(central vein), residual lipid vacuole (yellow arrow), kupffer cells (green arrow), regenerated hepatocytes (blue arrow). Magnification power 200 x.

4.Discussion:

Obese rats fed high fat diet for 12 weeks characterized by Leptin resistance that indicating from increasing caloric intake with high body weight (large fat from AI) associated with higher levels of plasma Leptin and Insulin concentrations. Saturated fatty acids had low oxidative rate (DeLany *et al.*,2000) which allowed to storage fat depots in adipose tissue that correlate positively with plasma Leptin causing higher Leptin levels (Sinitskaya *et al.*, 2007) in addition increased plasma lipid profile and triglycerides synthesis in the liver which in turn decreased LDL and VLDL clearance and increased their levels in the plasma (Grundy,1987). Saturated fatty acids caused change in composition of plasma membrane fatty acid that impaired Insulin signaling and reduced Insulin sensitivity (Storlien *et al.*, 1996).

Our results demonstrated that the obese rats treated with orlistat or metformin showed weight losing effect that reducing retroperitoneal adipose tissue (from AI) this may be related either to anorectic effect of the drug which reducing caloric intake (Hsieh *et al.*, 2005) or may related to decrease dietary fat absorption in the small intestine (Hollander *et al.*,1998)

The anorectic effect of metformin related to its action with Leptin in hypothalamus by increasing cerebello-spinal fluid Leptin concentration in both high fat obese rats and standard chow fed rats, or increased levels of STAT3 expression in

hypothalamus that responsible for Leptin action (kim *et al.*,2006).

Fasting plasma biochemical parameters were improved after treatment with anti-obesity drugs that may be related to the lipid –lowering effect through reduction of dietary fat absorption (Hsieh *et al.*, 2005) or fat storage in the body by reducing food intake.

The study of Hsieh *et al.*(2005) showed that orlistat had beneficial effects on LDL,TG and HDL concentration, while kim *et al*.(2006) reported that the biochemical parameters is decreased in metformin treated high fat fed obese rats.

The anti-obesity drugs were enhanced Leptin and Insulin sensitivity in high fat fed obese rats by decreased their plasma concentrations either by decreasing fat absorption and then fat storage which have positive correlation with plasma Leptin (Iossa *et al.*, 2003) or related to the effect of metformin on adipocyte signaling and endocrine function with robust inhibition of Leptin secretion via a mitogen activated protein kinase signaling pathway in brown adipocyte (Klein *et al.*, 2004). kim *et al.*(2006) showed that metformin correct Leptin resistance and induced Leptin sensitivity in obese rats by decreasing Insulin and Leptin levels.

The anti hyperglycemic effect of metformin drugs resulted from increasing levels of glucagon like peptide1 by inhibition of peptide degradation (Mannucci *et al.*, 2001). Metformin treatment caused reduction in fasting plasma glucose that related to decrease glucose production in liver by reduce the rate of gluconeogenesis (Hundal *et al*., 2000) and inhibit hepatic glycogenolysis (Cusi *et al*., 1996) or related to inhibition the activity of pyruvate carboxylase-phosphoenol pyruvate carboxykinase and increased conversion of pyruvate to alanine (Large & Beylot, 1999). Decreasing in concentration of HOMI (that reflect hepatic sensitivity to Insulin action) improve the action of plasma glucose and Insulin. The study of Yanovski *et al.* (2011) referred that metformin improve fasting plasma glucose, Insulin and HOMI in obese Insulin resistance children.

The elevation levels of liver enzymes ALT and AST in high fat fed group may be related to the direct association between high fat diet and hepatic steatosis (Carmiel-Haggai *et al.*, 2004) or may to increased saturated fatty acids in the liver that reflect liver injury by increasing levels of these enzymes (Wang *et al.*, 2006). Also excessive release of lipid from hepatocytes (Wanless & Shiota, 2004) had chemo-tactic activity for interleukin-8 which promoting tissue inflammation (Curzio & Esterbaucr 1995).

In the present study the high fat fed obese rats treated with anti-obesity drugs were effective to reduced hepatic steatosis and improved clinical parameters of NAFLD ALT and AST enzymes compared to placebo group.

Treated with orlistat showed mild reduction in liver steatosis this may be related to reduce in fat absorption in the small intestine, since orlistat inhibit gastric and pancreatic lipase and blocking fat and cholesterol absorption (Sabuncu *et al*., 2003). Previous researches referred to the beneficial effect of orlistat treatment to reduce liver enzymes levels and improve liver steatosis using ultrasound scan (Harrison *et al.*, 2004; Hatzitolios *et al*., 2004).

The short-term treatment with metformin was efficient to promote improvement in NAFLD and decreased liver steatosis, this effect related to either inhibition glucose absorption and improve Insulin sensitivity in peripheral target tissues (Hundal & Inzucchi, 2003; Fu *et al*., 2007) or related to activation effect of drug on adenosine monophosphate AMP protein kinase in hepatocyte that reduced acetyl-COA carboxylase (ACC) that induced fatty acids oxidation and suppressed sterol regulatory element binding protein (SREBP1) expression which led to inhibit glucose production and decreased plasma glucose and fatty liver (Zhou *et al*., 2001), or resulted from decrease plasma fatty acids that contributed to diminish lipid accumulation in liver tissue and reduce the rate of gluconeogenesis (Sindelar *et al*., 1997; Bergman & Mittelman , 1998). Metformin treatment showed significant reduction in liver enzymes ALT and AST, this finding was reported by other researchers (Chavez-Tapia *et al*., 2006; Adams & Angulo, 2006; Li *et al*., 2013). In conclusion the anti-obesity drugs showed favorable effect on body weight, lipid and lipoprotein concentrations also enhance Leptin and Insulin actions in obese rats fed high fat diets in addition to improve liver function and histological response in NAFLD.

Acknowledgement:
Author is grateful to Biology department - college of Science- Basrah to carry out this work.

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3/30/2014