

## A Comparative Study on the Effect of Leptin Hormone and Gemfibrozil in an Experimental Model of Hyperlipidemia Induced by Chronic Ethanol Treatment

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### ABSTRACT

The aim of the present study was to evaluate the effect of leptin hormone and Gemfibrozil on the body weight, hepatic & serum lipids and lipoproteins profile in ethanol-induced hyperlipidemia in rats. The study was carried on 53 male albino rats weighing 130-160 g classified into six groups (from A-F). Three of these groups were fed a normal diet (A, C and D), while the other groups (B, E and F) were fed a normal diet combined with ethanol (6.32 g/kg body weight per oral) for the first 30 days. Subsequently, the first three groups received a normal diet for group (A), in addition to Gemfibrozil (100 mg/kg per oral daily) for group (C) or exogenous leptin (230 µg/kg body weight, i.p.) every alternate day for group (D), while Groups (E) and (F) were administered Gemfibrozil and leptin respectively for the next 15 days. At the end of the total experimental period of 45 days, liver total lipids, serum concentrations of total cholesterol, HDL-C, LDL-C, VLDL-C, triglycerides, total proteins, albumin and glucose were measured. Ethanol-induced hyperlipidemia in rats resulted in marked increase of liver total lipids and significant increase of serum total cholesterol, LDL-C, VLDL-C and triglycerides levels. This was associated with concomitant decrease in serum HDL-cholesterol and glucose levels as well as serum total proteins and albumin levels. However, no changes were observed in the body weight gain. Administration of leptin or Gemfibrozil separately or after ethanol-induced hyperlipidemia to rats was able to antagonize the ethanol-induced biochemical changes in the tissues studied. The results of the current study showed that leptin administered alone to rats resulted in marked decrease of their body weight and fasting serum glucose levels while serum HDL-C was elevated. These findings indicated that the chronic administration of exogenous leptin was more effective as compared to Gemfibrozil in preventing the rise in lipids and lipoproteins concentration in an animal model of alcohol-induced hyperlipidemia.

**Keywords:** Alcohol, Leptin, Gemfibrozil, Hyperlipidemia, Lipoproteins, Proteins.

### INTRODUCTION

Leptin is a circulating hormone (16 kDa) produced and released primarily by adipocytes, it exerts a regulatory control on food intake and

energy expenditure<sup>(1)</sup>. Plasma leptin concentrations are correlated with total fat mass, percent body fat and body mass index (BMI). Leptin acts as a sensing hormone or 'lipostat' in a negative feedback control from

adipose tissue to the hypothalamus<sup>(2)</sup>. Thus, leptin informs the brain about the abundance of body fat, thereby allowing feeding behavior, metabolism and endocrine physiology to be coupled to the nutritional state of the organism.

Fibrates constitute a well known group of hypolipidemic drugs which are used in treatment of mixed hyperlipoproteinemia and hypertriglyceridemia<sup>(3)</sup>.

Gemfibrozil (GFZ) is a lipid regulating agent belonging to fibrates<sup>(4)</sup>. It was introduced in the 1980s, and was, also, supported by the published results of the Helsinki heart Study which showed a significant reduction in coronary heart disease (CHD) events and an acceptable safety profile<sup>(5)</sup>.

GFZ lowers plasma triglycerides and elevates HDL-C level in hypertriglyceridemic patients<sup>(6)</sup>.

Ethanol is a powerful inducer of hyperlipidemia in both animals and humans<sup>(7)</sup>. Also ethanol causes changes in the metabolism of lipoproteins<sup>(8)</sup>. Chronic alcohol intake is known to produce hypercholesterolemia, hyperlipidemia and hyper-triglyceridemia<sup>(9, 10)</sup>.

The aim of the present study was to evaluate the chronic effects of exogenous administration of recombinant leptin and GFZ in rats on serum, tissue lipids and lipoproteins in an animal model of alcohol-induced hyper-lipidemia.

## MATERIAL & METHODS

### 1-Material:

All chemicals used were of high analytical grade, products of Sigma

(USA) and El-Nasr Chemical Industries Company. Rat Recombinant Leptin Hormone was imported from Sigma (St.Louis USA) and Gemfibrozil (Parke –Davis USA) was from Alcan Pharma.

### 2-Animals and treatment:

In the present study, 53 male albino rats weighed 130-160 g, were classified into six groups (from A→F). Each group was composed of 8-10 rats and received normal diet during the total experimental period.

The first group (A) served as a normal control group receiving normal diet during the total experimental period of 45 days. While groups (C) and (D) received normal diet for 30 days. Liver cell damage was induced in rats of groups (B), (E) and (F) by administering 16% ethanol (6.32 g/Kg body weight) for 30 days. *The groups were treated as follows for the next 15 days:*

Groups (C) and (E) were administered Gemfibrozil (GFZ) (100 mg/Kg body weight per oral every day).

Groups (D) and (F) were administered exogenous leptin hormone (230 µg/Kg body weight i.p. every alternate day).

### 3-Biochemical analysis:

Animals were fasted overnight and the blood samples were withdrawn from retro-orbital sinus of all animals of different groups at the end of the experiment (total period of 45 days). Serum samples were separated and stored frozen -80°C until the following biochemical parameters were estimated: total cholesterol according to the enzymatic-colorimetric, Trinder, end point method as described by<sup>(11,12)</sup>, triglycerides according to the

enzymatic-colorimetric, end point method as described by<sup>(12,13)</sup>, low density lipoproteins (LDL-C) according to the precipitation, heparin/citrate method as described by<sup>(14, 15, 16, 17)</sup>, high density lipoproteins (HDL-C) according to the precipitation, phosphotungstic acid method as described by<sup>(18,19,20)</sup>, very low density lipoproteins (VLDL), total proteins according to the biuret reaction, End point method as described by<sup>(12,21,22,23)</sup>, albumin according to the colorimetric bromocresol green (BCG) method<sup>(12,23,24,25,26)</sup> and glucose according to the enzymatic-colorimetric, end point method as described by<sup>(12,27,28,29,30)</sup>.

At the end of the experiment (total period of 45 days), liver of all animals was separated after sacrifice and divided into two parts, the first for determination of total lipids according to the sulpho phosphovanilline method as described by<sup>(31)</sup> and the other for histopathological examination which was done as follows:

Liver slices (1 mm X 1 mm) were fixated in glutaraldehyde over night. Ultra thin sections of the liver were processed as blocks of pure resin following the standard micro technique and evaluated for histopathological changes under an electron microscope.

#### **4- Statistical analysis:**

Statistical analysis of the data was performed using ordinary one way analysis of variance (ANOVA) for parametric data followed by Tukey-Kramer multiple comparisons test or ANOVA for non-parametric data followed by Dunn's test<sup>(32)</sup>.

## **RESULTS**

### **1- Body weight gain:**

The body weight of ethanol-treated group showed a significant reduction as compared to normal control group. Gemfibrozil (GFZ) treatment to rats alone didn't show any change in their body weights when compared to normal controls.

In contrast, the GFZ treatment to ethanol-treated rats resulted in significant reduction of body weights of rats when compared with either normal controls or GFZ group. Leptin administration alone to rats showed a significant reduction in the body weights of rats as compared to normal controls, while, administration of leptin to ethanol-treated rats didn't show any significant differences from the leptin administered group [Table (1) and fig (1, 2)].

### **2- Liver total lipids, serum TG and VLDL-C:**

Table (2) and fig (3) shows that liver total lipids level was markedly increased in ethanol-treated group as compared to normal control group.

Administration of either GFZ or leptin alone to rats showed insignificant decrease in the liver total lipids level of rats as compared to normal control group, while they showed a marked reduction in liver total lipids as compared to ethanol-treated rats. On the other hand, administration of either GFZ or leptin to ethanol-treated rats showed a significant reduction in the liver total lipids as compared to ethanol-treated rats but still the values were higher than the normal control group.

The serum TG and VLDL-C levels of ethanol-treated group were significantly increased when compared to normal controls. Administration of either GFZ or leptin alone to normal rats didn't show any changes in their serum levels of TG and VLDL-C from the control values.

In contrast, administration of either GFZ or leptin to ethanol-treated rats produced marked reduction in serum TG and VLDL-C levels as compared to ethanol-treated rats.

**3- Serum total cholesterol (TC), LDL-C, HDL-C and LDL/HDL ratio:**

The serum levels of TC, LDL-C and LDL-C/HDL-C ratio were significantly elevated, while the serum level of HDL-C was significantly decreased in ethanol-treated group as compared to normal control group as given in table (3) and fig (4).

Administration of either GFZ or leptin alone to rats didn't change the level of serum studied parameters.

However, administration of GFZ or leptin to ethanol-treated groups resulted in significant reduction of the elevated serum level of TC, LDL-C and LDL/HDL ratio, while, the serum level of HDL-C was elevated.

**4- Serum total proteins, albumin and A/G ratio:**

Table (4) and fig (5) revealed that the serum albumin level of ethanol-treated group was significantly reduced as compared to normal controls, while the change in serum levels of total protein and A/G ratio of ethanol-treated group was

insignificant from the normal control values.

On the other hand, administration of either GFZ or leptin alone to rats didn't show any significant difference in the serum level of total protein, albumin and A/G ratio as compared to normal controls.

However, administration of either GFZ or leptin to ethanol-treated rats resulted in normalization of the serum levels of total proteins, albumin and A/G ratio.

**5- Serum glucose level:**

As shown in table (5) and fig (6), the serum glucose level of all groups didn't show any significant changes when compared to normal controls.

In contrast, leptin group showed a significant reduction in the serum glucose level when compared with either normal controls or ethanol-treated group.

**6- Histopathological examination of liver tissues:**

Fig (7) showed that in control rats the liver cells appeared with normal nature of cell organelles (fig 7a). While the liver cells of ethanol treated rats appeared unhealthy with slight congestion and fatty degeneration in portal canals (fig 7b).

Liver cells treated with GFZ were slightly improved with nearly normal nuclei and slight dilatation in endoplasmic reticulum (fig 7c). While leptin treated liver cells appeared normal with normal arrangement of plates and normal cell organelles (fig 7d).

Table (1): The effect of ethanol, GFZ, leptin hormone or combination of ethanol with either GFZ or leptin hormone on weight of animals (Mean  $\pm$  S.E):

Parameter \ Group	Control Group (A)	Ethanol Group (B)	GFZ Group (C)	Leptin Group (D)	Eth+GFZ Group (E)	Eth+Leptin Group (F)	
Parameter	Group	Control Group (A)	Ethanol Group (B)	GFZ Group (C)	Leptin Group (D)	Eth+GFZ Group (E)	Eth+Leptin Group (F)
Mean weight (g) of the animals at the start.	143.62 $\pm$ 2.94	141.20 $\pm$ 3.09	146 $\pm$ 3.55	147.50 $\pm$ 3	145.2 $\pm$ 3.46	150.13 $\pm$ 2.75	
Mean weight (g) of the animals at the end.	*** 165.62 $\pm$ 4.14	* 124.70 $\pm$ 4.74	158.40 $\pm$ 6.66	** 128.88 $\pm$ 4.06	* 112.20 $\pm$ 12.41	** 132.63 $\pm$ 4.26	
Weight (g) gain during the experiment	22 $\pm$ 1.19	xxx -16.50 $\pm$ 1.65 ccc	aaa 12.40 $\pm$ 3.11 bbb	xxx -18.62 $\pm$ 1.06	Xxx -33 $\pm$ 8.95 ccc	xxx -17.50 $\pm$ 1.51	

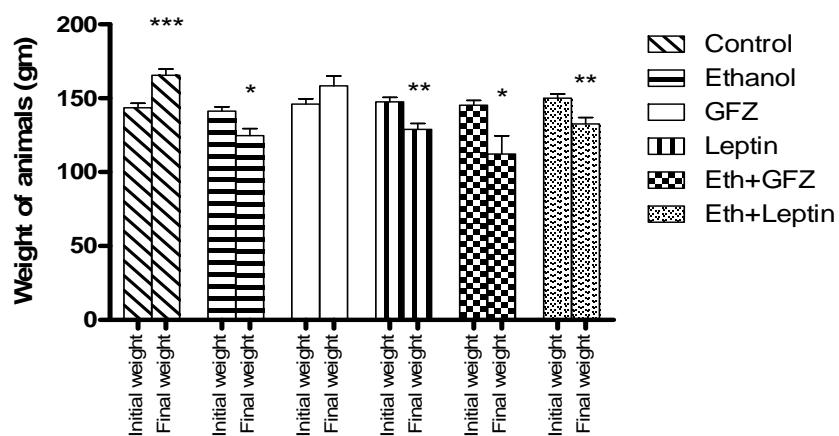
x, xx and xxx: Significantly different from normal control group at x=P<0.05, xx= P<0.01 and xxx= P<0.001.

(aaa): Significantly different from (Ethanol +Leptin) group at P=<0.001.

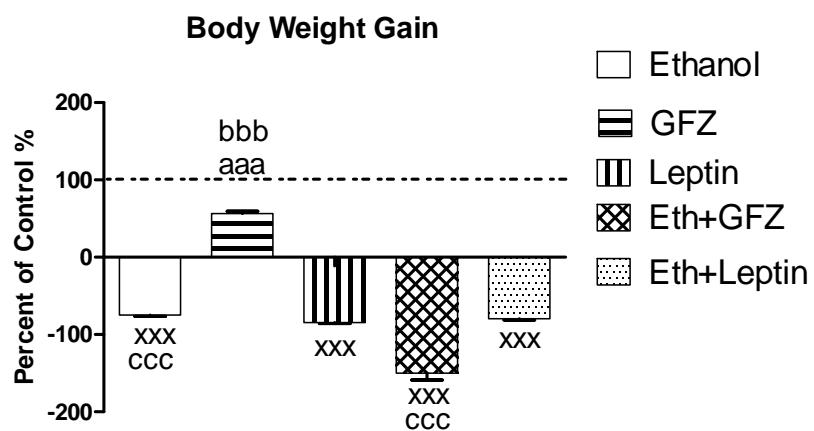
(bbb) Significantly different from (Leptin) group at P=<0.001.

(ccc): significantly different from (GFZ) group at P=<0.001.

\* , \*\* and \*\*\*: Significantly different from the corresponding group at initial weight at \* =P<0.05, \*\*=P<0.01 and \*\*\*P<0.001.



**Fig (1): Initial and final body weight of control and experimental rats administered with ethanol, GFZ, leptin, Ethanol+ GFZ and ethanol+ leptin for 45 days.**



**Fig (2): Weight gain of control and experimental rats administered with ethanol, GFZ, leptin, Ethanol+ GFZ and ethanol+ leptin for 45 days.**

Table (2): The effect of ethanol, GFZ, leptin hormone or combination of ethanol with either GFZ or leptin hormone on liver tissue total lipids, serum triglycerides and serum VLDL-C (Mean $\pm$ S.E):

Parameter \ Group	Control Group (A)	Ethanol Group (B)	GFZ Group (C)	Leptin Group (D)	Eth+GFZ Group (E)	Eth+Leptin Group (F)
Total Lipids in liver tissue(mg/dl)	608.33 $\pm$ 60.98	1336.68 $\pm$ 111.05 xx	476.67 $\pm$ 23.85 (aa)	645.92 $\pm$ 33.05	766.70 $\pm$ 135.28	<b>1015.86<math>\pm</math> 46.05</b>
Serum Triglycerides (mg/dl)	27.52 $\pm$ 3.05	129 $\pm$ 3.16 xxx	27.73 $\pm$ 3.02	31.91 $\pm$ 3.37	42.63 $\pm$ 8.58	<b>36.58<math>\pm</math> 3.34</b>
VLDL-Cholesterol (mg/dl)	<b>5.50<math>\pm</math> 0.61</b>	<b>25.80<math>\pm</math> 0.63</b> xxx	<b>5.54<math>\pm</math> 0.60</b>	<b>6.38<math>\pm</math> 0.44</b>	<b>8.53<math>\pm</math> 1.71</b>	<b>7.31<math>\pm</math> 0.66</b>

xx and xxx P<0.001: Significantly different from normal control group at xx= P<0.01 and xxx= P<0.001.

\*, \*\* and \*\*\*: Significantly different from ethanol-treated group (B) at \*=P<0.05, \*\*=P<0.01 and \*\*\*P<0.001.

(aa): Significantly different from (Ethanol +Leptin) group at P=<0.01.

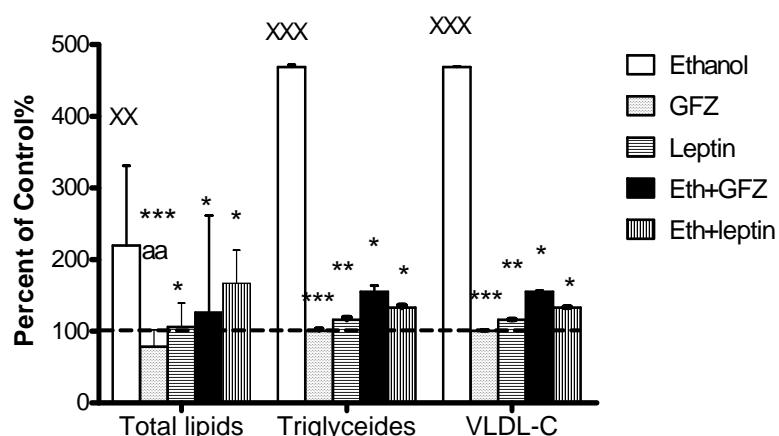


Fig (3): Effect of ethanol and either GFZ or leptin separately or combined with ethanol on liver total lipids, serum triglycerides and VLDL-C of control and experimental rats.

Table (3): The effect of ethanol, GFZ, leptin hormone or combination of ethanol with either GFZ or leptin hormone on serum level of total cholesterol and serum lipoproteins (HDL-C, LDL-C and LDL-C/HDL-C ratio) (Mean  $\pm$  S.E):

Parameter \ Group	Control Group (A)	Ethanol Group (B)	GFZ Group (C)	Leptin Group (D)	Eth+GFZ Group (E)	Eth+Leptin Group (F)
Serum Total Cholesterol (mg/dl)	68.96 $\pm$ 1.58	110.79 $\pm$ 2.69 xxx	70.91 $\pm$ 2.22 (aaa)	71.40 $\pm$ 3.25 (aaa)	73.35 $\pm$ 2.91 (aa)	90.01 $\pm$ 3.70 xxx
LDL- C (mg/dl)	32.62 $\pm$ 1.55	46.89 $\pm$ 2.76 xx	32.09 $\pm$ 1.63	32.30 $\pm$ 1.74	34.89 $\pm$ 2.63	32.62 $\pm$ 4.64
HDL-C (mg/dl)	33.10 $\pm$ 0.70	21.45 $\pm$ 2.14 xx	30.87 $\pm$ 0.78	37.31 $\pm$ 2.57 b	28.65 $\pm$ 0.58	34.03 $\pm$ 1.50
LDL-C /HDL-C ratio	0.98 $\pm$ 0.05	2.27 $\pm$ 0.13 xxx	1.04 $\pm$ 0.06	0.93 $\pm$ 0.06	1.22 $\pm$ 0.11	0.93 $\pm$ 0.12

xx and xxx P<0.001: Significantly different from normal control group at xx= P<0.01 and xxx= P<0.001.

\*, \*\* and \*\*\*: Significantly different from ethanol-treated group (B) at \*=P<0.05, \*\*=P<0.01 and \*\*\*P<0.001.

(aa) and (aaa): Significantly different from (Ethanol +Leptin) group at aa=P<0.01 and aaa= P<0.001.

(b): Significantly different from (Leptin) group at P=<0.05.

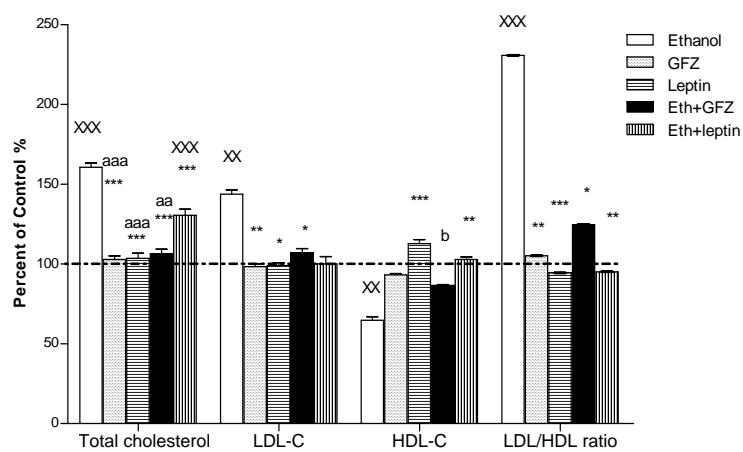


Fig (4): Effect of ethanol and either GFZ or leptin separately or combined with ethanol on serum total cholesterol, LDL-C, HDL-C and LDL-C/HDL-C ratio levels of control and experimental rats.

Table (4): The effect of ethanol, GFZ, leptin hormone or combination of ethanol with either GFZ or leptin hormone on serum level of total protein, albumin and albumin globulin ratio (A/G ratio) (Mean $\pm$ S.E):

Parameter \ Group	Control Group (A)	Ethanol Group (B)	GFZ Group (C)	Leptin Group (D)	Eth+GFZ Group (E)	Eth+Leptin Group (F)
Serum Total Proteins (g/dl)	6.78 $\pm$ 0.07	5.24 $\pm$ 0.22	8.08 $\pm$ 0.44 aaa	7.35 $\pm$ 0.28 a	6.71 $\pm$ 0.10	<b>6<math>\pm</math>0.24</b>
Serum Albumin (g/dl)	4.45 $\pm$ 0.07	3.45 $\pm$ 0.05 xxx	4.61 $\pm$ 0.07	4.11 $\pm$ 0.36	4.12 $\pm$ 0.05	<b>4.22<math>\pm</math> 0.08</b>
A/G ratio	1.83 $\pm$ 0.10	1.93 $\pm$ 0.18	1.43 $\pm$ 0.10 a	1.44 $\pm$ 0.30 a	1.60 $\pm$ 0.05	<b>2.97<math>\pm</math> 0.56</b>

xxx: P<0.001: Significantly different from normal control group at P=<0.001.

\* and \*\*\*: Significantly different from ethanol-treated group (B) at \* = P<0.05 and \*\*\*P<0.001.

(a) and (aaa): Significantly different from (Ethanol +Leptin) group at aa=P<0.05 and aaa= P<0.001.

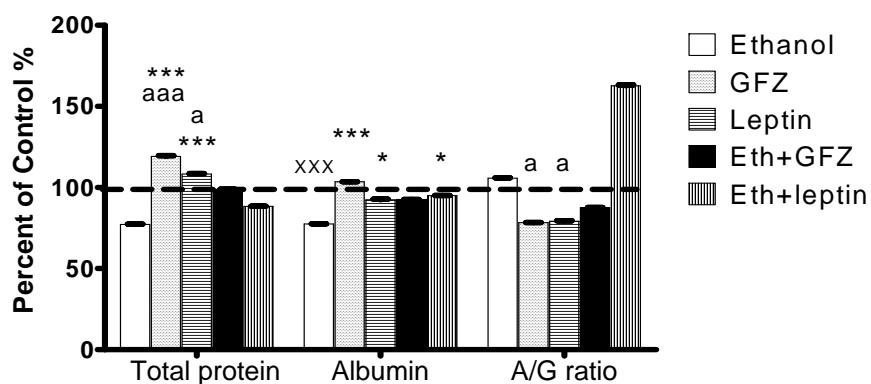


Fig (5): Effect of ethanol and either GFZ or leptin separately or combined with ethanol on serum total protein, serum albumin and A/G ratio levels of control and experimental rats.

Table (5): The effect of ethanol, GFZ, leptin hormone or combination of ethanol with either GFZ or leptin hormone on serum level of glucose (Mean $\pm$ S.E):

Parameter \ Group	Group (A) Control	Group (B) Ethanol	Group (C) GFZ	Group (D) Leptin	Group (E) Eth+GFZ	Group (F) Eth+Leptin
Serum level of glucose (mg/dl)	108.45 $\pm$ 1.71	97.68 $\pm$ 6.19	102.86 $\pm$ 4.52 b	77.08 $\pm$ 2.85 ** a	84.21 $\pm$ 3.77 a	108.65 $\pm$ 17.90

\*\*: Significantly different from ethanol-treated group (B) at P=<0.01.

(a): Significantly different from (Ethanol +Leptin) group at P=<0.05.

(b): Significantly different from (Leptin) group at P=<0.05.

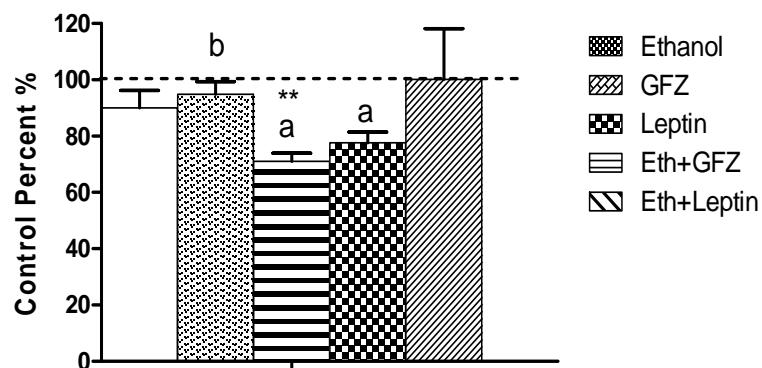
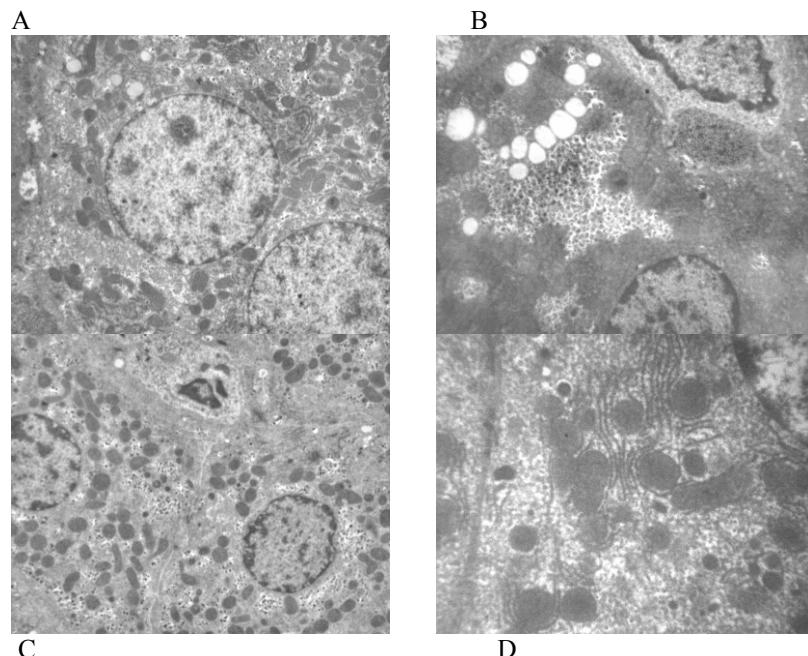


Fig (6): Effect of ethanol and either GFZ or leptin separately or combined with ethanol on serum glucose level of control and experimental rats.



**Fig(7): Representative photomicrographs of the histopathological changes in the liver of rats after administering ethanol and either GFZ or leptin separately or combined with ethanol (A) Control rat, (B) Ethanol treated rat, (C) GFZ treated rat, (D) Leptin treated rat.**

## DISCUSSION

Alcoholism is a major problem in the modern world<sup>(33)</sup>. The World Health Organization (WHO) announced that alcohol consumption is responsible for increasing the risk of liver cirrhosis, certain cancers, raised blood pressure, stroke and congenital malformations<sup>(34)</sup>. Alcohol abuse is now becoming a rising problem in the Middle East due to globalization.

Interaction of ethanol with lipid metabolism is complex. When ethanol is present, it becomes the preferred fuel for the liver and displaces fat as a

source of energy, blocking fat oxidation and favoring fat accumulation. The accumulation of fat in the liver on chronic alcohol intake acts as a stimulus for the secretion of lipoproteins into the blood stream and the development of hyperlipidemia<sup>(35)</sup>.

Leptin hormone was discovered in 1994, it is secreted mainly by adipocytes, although expression in placenta, fetal tissue, stomach and other tissues have also been observed. Leptin informs the brain about the size of the fat stores<sup>(36)</sup>, and has a wide variety of central and peripheral actions, including effects on reproduction, lipid metabolism,

immune system, blood pressure and angiogenesis<sup>(37)</sup>.

In the present study, the difference in weight of rats at the end of the experiment in groups B, D & F were significantly lower than at the beginning of the experiment. These results agreed with those of *Kalaivanisailaja et al*<sup>(38)</sup> and *Balasubramaniyan et al*<sup>(39)</sup> and this was attributed to alcohol induction to diuresis<sup>(40,41)</sup>, malabsorption of calories<sup>(42)</sup> and increase in oxygen consumption which may lead to weight loss<sup>(43)</sup>.

Leptin administration alone or mixed with ethanol was shown to prevent further weight gain of the animals<sup>(39,44)</sup>. Investigators have traced the neuronal pathways through which leptin works in the brain and have shown that other hormones involved in weight control, work through the same pathways especially the arcuate nucleus<sup>(45)</sup>. Leptin acts through the arcuate nucleus and increases the expression of cortisol releasing factor (CRF), decreases neuropeptide Y (NPY) and affects the expression of other neuropeptides involved in appetite and energy balance. These effects strongly implicate leptin as a negative feedback signal, which reflects body adiposity<sup>(37)</sup>.

The present study, ethanol treated group resulted in significant elevation of liver total lipids as compared to normal control group. This finding is consistent with the report of *Kalaivanisailaja et al*<sup>(38)</sup>. Treatment of the animals with GFZ or leptin alone or mixed with ethanol resulted in significant reduction of liver total lipids as compared to ethanol induced-

hyperlipidemia group. Leptin is known to regulate lipid metabolism both indirectly through the CNS and directly in the peripheral tissues. Both pathways end up with similar outcomes, a decrease in triglycerides (TG) synthesis and increase in lipolytic rates and lipid oxidation. This is achieved by a reduction in calorie intake, causing increased reliance on internal energy stores, mainly TG. Modulation of energy expenditure by leptin through inhibition of metabolic rate, essentially acts to further increase TG use. This shift in metabolism towards a catabolic route coupled with the direct lipolytic and oxidative effects of leptin on peripheral tissues sum together to cause more rapid utilization of TG stores<sup>(46)</sup>.

It was reported that GFZ increases liver expression and binding activity of peroxisome proliferator-activated receptor (PPAR $\alpha$ ) and it has been shown PPAR $\alpha$  activity increases liver X receptor (LXR) expression and the activity of that receptor is directly involved in the control of lipid and glucose metabolism<sup>(47)</sup>.

In the present study, the mean serum levels of total cholesterol (TC) and TG were elevated significantly in ethanol treated group in agreement with *Balasubramaniyan et al*<sup>(44)</sup>.

The increased intracellular accumulation of TG in the presence of ethanol is due to increased fatty acid uptake, decreased fatty acid oxidation in the citric acid cycle (TCA) and decreased lipoprotein secretion and decreased activity of lipoprotein-lipase<sup>(48)</sup>.

Administering leptin alone or mixed with ethanol markedly reduced

both TC and TG. This is consistent with *Kalaivanisailaja et al*<sup>(38)</sup> and *Balasubramaniyan et al*<sup>(39,44)</sup>.

*Cohen et al*<sup>(49)</sup> demonstrated that leptin may attenuate insulin activity in isolated hepatocytes. This may explain the cause for the normalization of serum TC level observed in leptin-treated mice. However, reduction in lipogenesis, decreased tissue TG content and decreased glucose oxidation are consistent with the hypothesis that leptin modulates energy homeostasis by directing lipids away from storage to oxidation, as suggested by in vitro studies<sup>(50)</sup>. The TG content of islets is an important modulator of insulin secretion<sup>(51)</sup> and leptin has been shown to deplete islets TG content<sup>(52)</sup> and increase the expression of the enzymes involved in fatty acid oxidation<sup>(53)</sup>.

GFZ was found to lower plasma TG level in patients and animal models<sup>(54)</sup> through its effect on PPAR $\alpha$ . PPAR $\alpha$  mediates regulation of several genes involved in metabolism of triglycerides rich lipoprotein. This may in turn lead to: increased lipoprotein lipase (LPL) mediated lipolysis and lowered hepatic apolipoprotein C-III synthesis<sup>(55)</sup>.

In the present study ethanol-induced hyperlipidemia group showed significant reduction in high density lipoproteins (HDL-C) serum level and at the same time significant elevation in low density lipoproteins (LDL-C), very low density lipoproteins (VLDL-C) serum levels and LDL-C/HDL-C ratio as compared to normal control group. Ethanol consumption leads to increased concentrations of plasma

LDL-C and VLDL-C, as the release of these lipoproteins from the liver apparently does not keep pace with the rate of formation of TG which accumulates in the liver in addition to decrease in activity of LPL<sup>(56)</sup>. The lowered HDL-C levels in mice could be attributed to decreased plasma LPL and lecithin cholesterol acyl transferase (LCAT) activities in these mice<sup>(38)</sup>.

*Silver et al*<sup>(57)</sup> demonstrated that hepatocytes of ob/ob mice (mutant leptin gene) had reduced binding, association, degradation, recycling and resecretion of HDL apoproteins and this defect in the liver catabolism of HDL was reversed by low-dose of leptin treatment.

In the current study, leptin administration alone or mixed with ethanol showed marked improvement in HDL-C, LDL-C, VLDL-C and LDL-C/HDL-C ratio compared to ethanol treated rats. Leptin administration resulted in the optimum activity of plasma LPL which may lead to decrease in LDL-C and VLDL-C levels<sup>(38)</sup>.

In the current study, the mean values of serum glucose level of all groups were lower but not significantly than that of control group. Treatment of leptin alone showed significant reduction in serum glucose level as compared to ethanol-induced hyperlipidemia group.

Low-dose leptin treatment ameliorates insulin resistance and hyperlipidemia in patients with low leptin levels resulting from congenital or acquired lipodystrophy<sup>(58)</sup>. Metabolically, leptin promotes the redistribution of intrahepatic glucose fluxes with an increase in

gluconeogenesis and a parallel decrease in glycogenolysis<sup>(59,60)</sup>. It was confirmed that leptin causes an improvement in skeletal muscle insulin sensitivity by both peripheral and central effect<sup>(61)</sup> that fully agrees with the present results.

In contrast to the current results, some studies reported that leptin inhibits insulin gene transcription<sup>(62,63)</sup> and insulin secretion<sup>(64,65)</sup>.

Leptin can inhibit insulin secretion by activating ATP-dependent potassium channels or via interactions with the cAMP protein kinase A signaling pathway<sup>(66)</sup>, perhaps by activating phosphodiesterase B<sub>3</sub><sup>(67)</sup>.

**Dumont et al** <sup>(68)</sup> suggested that insulin resistance may not be improved without major variation in plasma TG levels nor in body fatness and glucose level in GFZ treated rats. On the other hand, Steiner <sup>(69)</sup> had already reported improved insulin sensitivity in non-diabetic subjects treated with GFZ.

In the present study administering leptin to ethanol-administered rats significantly reduced the fatty changes and improved liver tissue histology which is agreed with the results of **Kalaivanisailaja et al.**<sup>(38)</sup>. Acetaldehyde formed by the enzymatic oxidation of alcohol in the liver is thought to play a major role in the development of cell damage from alcohol. It may combine covalently with cell membrane proteins causing membrane damage. It may, also, damage hepatic microtubules by interfering with the liver proteins and promoting collagen formation and, also, damage hepatic mitochondria<sup>(70)</sup>. Administering leptin to ethanol

administered rats significantly reduced the fatty changes and improved liver tissue histology which proves that elevated systemic leptin levels can attenuate accumulation of fat in the liver, preventing lipoapoptosis and our results are fully agreed with **Kalaivanisailaja et al** <sup>(38)</sup>.

## CONCLUSION

In conclusion, the findings of the present study indicated that intraperitoneal injection of leptin hormone was more effective as compared to GFZ in preventing the rise in lipids and lipoproteins concentrations significantly in an animal model of alcohol induced hyperlipidemia. Moreover, the histopathological examinations revealed that leptin treatment had more protective effects on rat's liver in comparison to GFZ treatment.

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## دراسة مقارنة حول تأثير هرمون الليتين و جيفيبروزيل في الفئران المستحدث بها زيادة في الدهون بكمول الأيتيل

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تهدف هذه الدراسة الى تقييم تأثير هرمون الليتين و الجيفيبروزيل على وزن الجسم و مستوى الدهون و البروتينات الدهنية في دم و كبد فئران التجارب التي تم رفع مستوى الدهون فيها بكمول الأيتيل. أجريت هذه الدراسة على ثالث و خمسين فأر ذكور من السلالة البيضاء (التي تتراوح أوزانها من ١٣٠ - ١٦٠ جم) و الذين تم تقسيمهم الى ستة مجموعات من (A-F). ثلاثة من هذه المجموعات تم تغذيتها على علف عادي (A,C & D) و المجموعات الثلاثة الأخرى (B,E&F) تم تغذيتها على العلف بالإضافة الى كمول الأيتيل (٦٢،٦ جم/كجم من وزن الفأر عن طريق الفم) لمدة شهر. وبعد ذلك استمرت مجموعة (A) في التغذية على العلف العادي، مجموعة (C) تم اعطائها جيفيبروزيل (١٠٠ مجم /كجم من وزن الفأر عن طريق الفم يوميا) و مجموعة (D) تم اعطائها هرمون الليتين (٢٣٠ ميكروجرام/كجم من وزن الفأر عن طريق الحقن البريتوني يوم بعد يوم). أما بالنسبة للمجموعتين (E&F) تم اعطائهما جيفيبروزيل و هرمون الليتين على التوالي لمدة أسبوعين. في نهاية مدة هذه الدراسة (٤٥ يوما) تم قياس مستوى الدهون في الكبد و مستوى الكوليستيرول، الدهون الثلاثية، البروتينات الدهنية، البروتين الكلي، الألبومين و الجلوكوز في الدم. وقد لوحظ أن ارتفاع مستوى الدهون في دم الفئران المستحدث بكمول الأيتيل قد أدى إلى زيادة ملحوظة في كمية الدهون في الكبد بالإضافة إلى زيادة الكوليستيرول الكلي، كوليستيرول البروتينات الدهنية منخفض الكثافة وكوليستيرول البروتينات الدهنية منخفض الكثافة جدا في الدم. هذا بالإضافة إلى انخفاض ملحوظ في الكوليستيرول البروتينات الدهنية عالي الكثافة و مستوى الجلوكوز و كذلك البروتين الكلي و الألبومين في الدم و لوحظ انخفاض في وزن الفأر. وقد لوحظ أن اعطاء هرمون الليتين أو جيفيبروزيل بمفردهم أو بعد ارتفاع مستوى الدهون في دم الفئران المستحدث بكمول الأيتيل كان كفياً بمعادلة تأثير التغيرات الحيوية في الأنسجة تحت الدراسة. كذلك أظهرت نتائج هذه الدراسة أن اعطاء هرمون الليتين بمفرده للفئران أدى إلى انخفاض ملحوظ في أوزان الفئران و مستوى الجلوكوز في الدم و في نفس الوقت ارتفاع مستوى كوليستيرول البروتينات الدهنية عالي الكثافة و الدهون الثلاثية في الدم. و يستخلص من نتائج هذه الدراسة أن اعطاء هرمون الليتين للفئران كان أكثر فاعلية مقارنة بالجيفيبروزيل في منع زيادة الدهون و البروتينات الدهنية في الفئران المستحدث بها زيادة في الدهون باستخدام كمول الأيتيل.