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Influence of Liraglutide Alone and in Combination with Glimepiride on Hepatic Function in Obese-Diabetic Rabbits

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The study was done to find out the effect of liraglutide alone and in combination with glimepiride on hepatic function in obese diabetic rabbits. Obesity was produced with HFD (High fat diet) given for 10 weeks and diabetes was induced with dithizone (5 mg/kg i.p.). All the obese-diabetic rabbits showed elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), BGLs (Blood glucose levels) and increased fatty infiltration of liver. Liraglutide (7µg/kg) s.c. decreased the elevated levels of AST, ALT, BGLs and reduced fatty infiltration of liver in obese-diabetic rabbits. However, combined effect of liraglutide and glimepiride (2mg/kg orally) failed to show any improvement of hepatic dysfunction rather worsened the impairment. It is therefore concluded that Liraglutide alone and not in combination with glimepiride is effective in improving the hepatic dysfunction of obese-diabetic rabbits.

Keyword: Liraglutide, Glimepiride, Hepatic Function, Obese-Diabetic Rabbits

1. Introduction

The rapid increase in the prevalence of obesity along with type 2 diabetes mellitus is a major global health problem and is known as 'diabesity' epidemic^[1]. These metabolic disorders may cause diseases like non-alcoholic fatty liver disease, gall bladder disease, osteoarthritis and some cancers^[2,3]. Diabetes mellitus and obesity have been associated with mild asymptomatic elevation in the serum levels of hepatic enzymes^[4,5]. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease usually associated with obesity, type 2 diabetes and hyperlipidemia^[6]. The increasing prevalence rate of abnormality is not very well understood and elevations in patients with obese diabetics are often attributed to fatty infiltration of liver and

this kind of fatty change or hepatic steatosis is strongly associated with the presence of diabetes and obesity. Histologically, NAFLD may manifest as hepatic steatosis or may be accompanied by hepatocellular damage with inflammation and/or fibrosis, which is termed non-alcoholic steatohepatitis (NASH)^[4]. Liver pathology will be similar to that seen in alcoholic liver disease starting initially with fatty infiltration then it can progress to focal and diffuse fibrosis, later ultimately ends up with cirrhosis^[4]. Hepatic enzymes will be elevated initially which will reflect the extent of liver injury and the likelihood of having an elevated serum alanine aminotransferase levels is greater among persons with type 2 diabetes those who are overweight or obese^[7]. Drug induced liver

diseases (DILD) mimic various forms of liver injury that range in severity from transient, asymptomatic elevation in ALT levels to fulminant liver failure. The diagnosis of DILD is predicated on the exclusion of other possible causes and on the identification of the pattern of liver test abnormality, the duration of latency to symptomatic presentation and the response to drug withdrawal^[8]. There is evidence of hepatotoxicity with oral anti diabetic drugs and has focused attention on hepatic function in the patients who take such drugs^[9]. Hepatic dysfunction has been reported with sulfonylureas^[10]. Glimepiride is the most recently and commonly prescribed sulfonylurea for the treatment of type 2 diabetes. It has been observed from some case reports indicating development of cholestatic hepatic injury in the patients treated with glimepiride^[10]. Liraglutide in combination with oral hypoglycemic drugs is effective in controlling hyperglycemia in type 2 diabetes. It is commonly combined with either glimepiride, metformin or other anti-diabetic drugs^[11]. Since there is a high prevalence of co-morbid hepatic impairment in obese-diabetic population, it is of clinical importance to realise the effects of liraglutide on hepatic function in these individuals^[12,13]. As of now there are limited studies with respect to the response of liraglutide along with glimepiride on hepatic function in obese diabetes. So the proposed study therefore, was designed to find out the influence of liraglutide alone and in combination with glimepiride on hepatic function in experimental obese diabetic rabbits.

2. Material and Methods

2.1 Animals

The study was conducted in New Zealand white adult rabbits weighing approximately 2-2.5 kg of either sex which were housed individually in standard cages at natural light/dark condition and room temperature maintained at 26±2 °C. The animals were obtained from Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar. They were provided with food and water ad libitum. The animals were acclimatized to the laboratory conditions for at least 7 days prior to

the experiments. After a week of acclimation period, the animals were fed with standard rabbit's chow having composition of all dietary elements appropriate for maintaining normal rabbit^[14]. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Sciences, Rohtak.

2.2 Experimental Obesity

To induce obesity, the rabbits were given high fat diet (HFD) constituting 10% fat (2/3 corn oil and 1/3 animal lard) to a standard normal rabbit chow for 10 weeks^[15]. Weekly body weight was recorded throughout the period of 10 weeks. Similarly, skin fold thickness (SFT) lateral to umbilicus, measured in mm was recorded. The body weight was recorded using a standard animal weighing machine and skin fold thickness was measured with the help of vernier caliper. The rabbits were considered obese which showed approximately 25% gain in body weight and 37% increase in skin fold thickness (SFT) at the end of 10 weeks of high fat diet intake^[14].

2.3 Experimental Diabetes

To induce diabetes, the rabbits were given single injection of dithizone 5mg/kg body weight intraperitoneally. Seventy two hours was allowed for full development of diabetes^[16]. Rabbits showing blood glucose levels ≥ 250 mg/dl were considered diabetic.

2.4 Laboratory Analysis of Biochemical Measures

Serum levels of hepatic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured adopting standard techniques available in the department of Biochemistry of this institution. These biochemical measures were done before the treatment followed by weekly for 3 weeks during the treatment and at the end of the treatment.

2.5 Histology of Liver

Liver tissue was taken with the help of liver biopsy needle under ketamine (20 mg/kg i.m.)

anesthesia. The histological study was done before and after the end of treatment.

2.6 Drugs and Chemicals

Liraglutide was purchased from Novo Nordisk, India Pvt. Limited, Bengaluru and was used in doses of 7µg/kg, s.c. Glimpiride obtained from Med Care Remedies Pvt. Ltd, Una, Himachal Pradesh and was used in doses of 2mg/rabbit/oral^[17]. Dithizone was purchased from Keminova India chemicals Pvt. Ltd. Thane, Mumbai and was used in doses of 5mg/kg, i.p. for inducing diabetes^[16]. Ketamine was obtained from the department of Anesthesia, Pt. B.D Sharma, PGIMS, Rohtak. Rabbit chow was purchased from the market.

2.7 Experimental Design and Protocol

The rabbits were divided into three pre-treatment categories having 24 rabbits in each group according to the presence of obesity and/or diabetes as follows:

- A. Rabbits with diabetes
- B. Rabbits with obesity
- C. Rabbits with obesity and diabetes.

Each above pre-treatment category were further sub-divided into four groups having 6 rabbits in each for the purpose of different drug treatment as follows:

Group 1. Rabbits received vehicles of inj. Liraglutide and /or Glimpiride daily for 3 weeks.

Group 2. Rabbits received inj. Liraglutide 7µg/kg/daily s.c. for 3 weeks.

Group 3. Rabbits received Glimpiride 2mg /Rabbit/daily orally for 3 weeks.

Group 4. Rabbits received inj. Liraglutide 7µg/kg/daily s.c. and Glimpiride 2mg /Rabbit/daily orally for 3 weeks.

Blood samples measuring 5ml was drawn from the marginal veins of rabbit ear for the biochemical analysis before and weekly during treatment and at the end of treatment.

2.8 Statistical Analysis

The data was collected from various study groups and expressed as Mean ± Standard Error of Mean (m ± SEM). The data were analyzed using Repeated Measures Analysis Of Variance (RM-ANOVA) with Bonferroni's correction. P value less than 0.05 was considered to be statistically significant. All statistical calculations were performed with SPSS software package.

3. Results

3.1 Effect of Liraglutide on hepatic enzymes, AST & ALT in obese-diabetics

All the obese-diabetic rabbits showed elevated levels of AST and ALT. Liraglutide (7µg/kg/day, s.c.) decreased the elevated levels of these enzymes. However, the decrease in the enzyme levels in all the obese-diabetic rabbits was not statistically significant (p>0.05) at day 21 as shown in Table 1.

Table 1: Effect of Liraglutide on AST and ALT in obese-diabetic rabbits

Pre-treatment Rabbits	AST (IU/L)			
	Baseline	7 th day	14 th day	21 st day
Diabetes	48.33±0.42	48.30±0.42	47.23±0.42	47.33±0.42
Obese	52.66±0.33	52.33±0.21	51.66±0.21	50.66±0.21
Obese Diabetes	74.83±0.83	74.78±0.83	73.75±0.83	72.83±0.83
Pre-treatment Rabbits	ALT (IU/L)			
	Baseline	7 th day	14 th day	21 st day
Diabetes	57.0±0.73	57.50±0.56	56.50±0.56	55.36±0.56
Obese	61.33±0.80	61.0±0.89	60.26±0.87	59.0±0.89
Obese Diabetes	89.16±1.42	88.33±1.49	87.34±1.49	87.10±1.49

All values are expressed as mean ± SEM

3.2 Effects of combination of Liraglutide and Glimpeiride on hepatic enzymes AST & ALT in obese-diabetics:

The combination of Liraglutide and Glimpeiride further increased the hepatic enzymes, AST and

ALT levels from the baseline in all obese-diabetic rabbits at the end of treatment (day 21) significantly ($p < 0.05$) as shown in Table 2.

Table 2:Effect of Liraglutide & Glimpeiride on AST & ALT in obese-diabetic rabbits

Pre-treatment Rabbits	AST(IU/L)			
	Baseline	7 th day	14 th day	21 st day
Diabetes	48.33±0.42	50.33±0.42	52.34±0.42*	54.52±0.42*
Obese	52.66±0.33	54.16±0.47	55.45±0.47	57.17±0.47*
Obese Diabetes	74.83±0.83	75.83±0.83	77.66±0.83	79.16±0.83*
Pre-treatment Rabbits	ALT(IU/L)			
	Baseline	7 th day	14 th day	21 st day
Diabetes	57.0±0.73	58.50±0.80	60.50±0.80	61.16±0.74*
Obese	61.33±0.80	62.83±0.79	64.84±0.79	66.82±0.79*
Obese Diabetes	89.16±1.42	89.54±1.35	91.55±1.35	93.39±1.35*

All values are expressed as mean ± SEM

* - Comparison between baseline AST & ALT values with values at 7th, 14th & 21st day in respective pre-treatment groups (p value < 0.05).

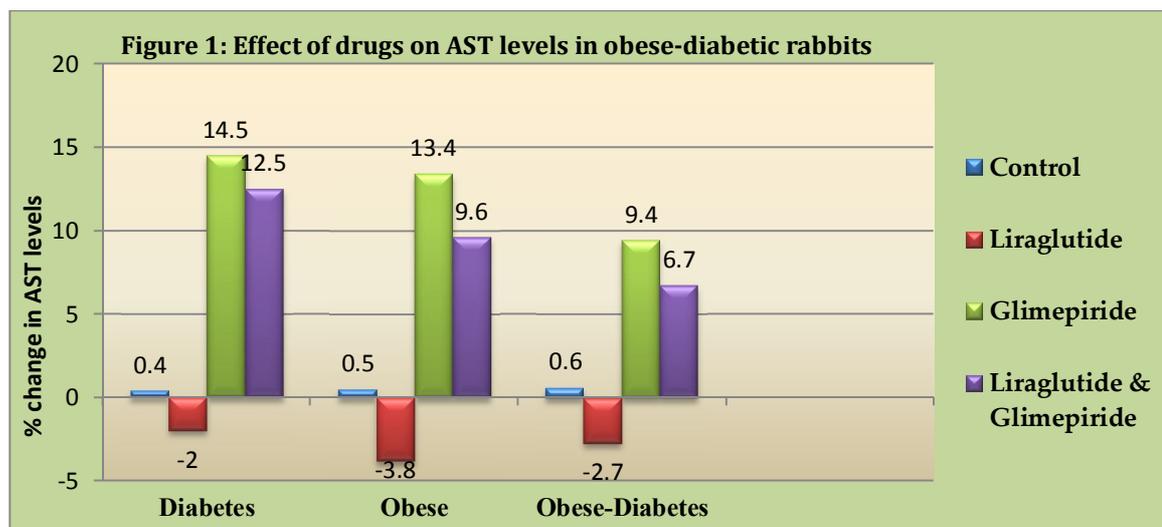


Fig 1: Effect of drugs on AST levels in obese-diabetic rabbits

3.3 The Percentage (%) Change in AST Levels

In obese-diabetic group the reduction in AST with liraglutide was 2.7%. However, with glimepiride and in combination with liraglutide the AST level was elevated by 9.4% and 6.7% respectively. Furthermore, Vehicle injected control group did not show any significant change as shown in figure1.

3.4 The percentage (%) change in ALT levels

In obese-diabetic group the reduction in ALT with liraglutide was 2.2%. However, with glimepiride and in combination with liraglutide the ALT level was elevated by 5.6% and 4.5% respectively. Furthermore, the vehicle treated control group did not show any significant change as shown in figure 2.

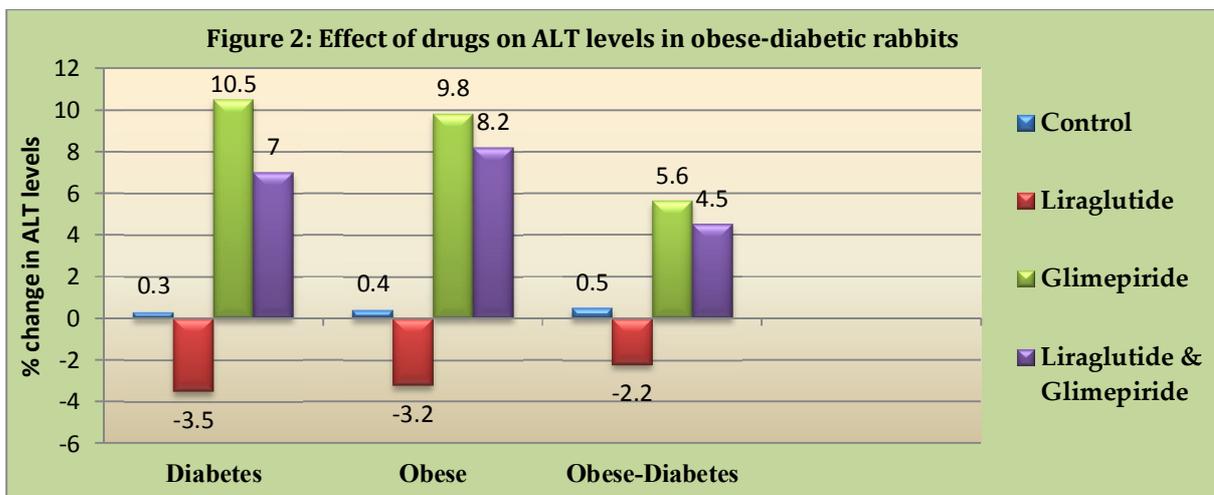
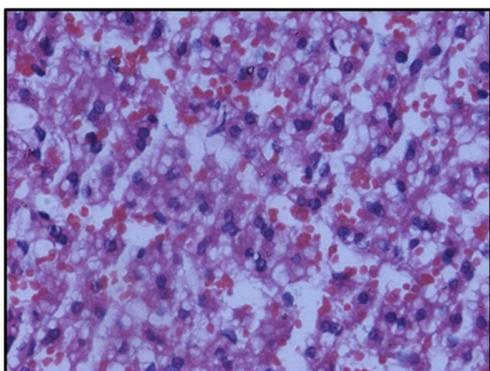


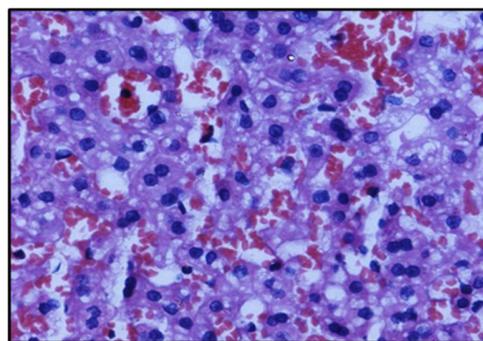
Fig 2: Effect of drugs on ALT levels in obese-diabetic rabbits

3.5 Histological Changes:

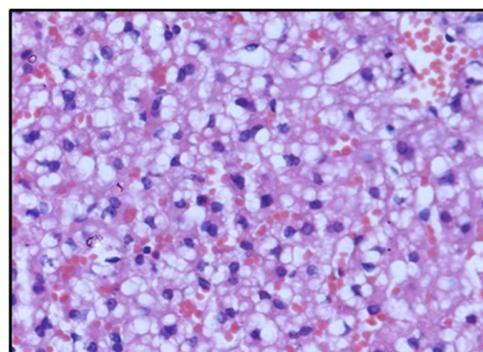
The effect of Liraglutide alone and in combination with Glimepiride in obese-diabetic rabbits was studied. Grade III fatty change seen in obese-diabetic rabbits (slide 1) which was reduced to grade II with liraglutide (slide 2). However, it remained as such without any significant change with the drug combination (slide 3).



Slide 1: obese-diabetic rabbit liver (Before treatment) (H & E, 100X) showing grade III fatty change along with fatty cyst and inflammatory infiltrate with sparse fibrosis in the interstitium.



Slide 2: Effect of liraglutide in obese-diabetic rabbit. (H & E, 100X) showing grade II fatty change along with fatty cyst and mild inflammatory infiltrate. Fatty change in liver reduced from grade III (earlier biopsy) to grade II (present biopsy).



Slide 3: Effect of liraglutide and glimepiride in obese diabetic rabbit (H & E, 100X) showing grade III fatty change along with mild inflammatory infiltrate in the liver parenchyma. Fatty change in liver remained as such without any significant change.

4. Discussion

Liraglutide is a novel human GLP-1analogue approved for treatment of type 2 diabetes mellitus. Diabetes along with obesity have been associated with asymptomatic elevation in the serum levels of hepatic enzymes^[10]. This is often attributed to fatty infiltration of liver. There is evidence of hepatotoxicity with oral anti-diabetic drugs and this has focused attention on hepatic function in the patients who take such drugs^[10]. Injury induced with sulfonylureas group of oral hypoglycaemic agents has been reported infrequently^[8]. However, there are some case reports indicating development of cholestatic hepatic injury in the patients treated with glimepiride^[10]. Since there is a high prevalence of co-morbid hepatic impairment in this population, it is essential to establish the effects of Liraglutide and in combination with glimepiride on hepatic function. In our study, it was observed that the elevation of hepatic enzymes, AST and ALT in obese rabbits were greater compared to diabetes. In diabetic rabbits the hepatic enzymes were raised significantly compared to normal. Moreover we found that the obese diabetic rabbits showed much more increase in AST and ALT levels compared to either obese or diabetic rabbits.

Liraglutide decreased the elevated levels of hepatic enzymes (AST and ALT) in almost all obese-diabetic rabbits but the reduction in enzyme levels was not significant ($p>0.05$). Paradoxically, it was interesting to observe that liraglutide when combined with glimepiride increased the hepatic enzymes significantly ($p<0.05$). This raised hepatic enzyme levels could be attributed to the unopposed effect of glimepiride on liver. These observations explain hepatoprotective action of liraglutide and it definitely did not allow glimepiride to exert its full effect on hepatic enzyme levels when given in combination. The exact cause of these effects is not clear. The probable reasons could be attributed to liraglutide which have the property to decrease the raised levels of blood glucose, triglyceride, total cholesterol and LDL counteracting the elevation of hepatic enzymes which occurred as a result of hepatic damage

caused by hyperglycaemia and hyperlipidaemia in obese-diabetics.

Flint *et al.*^[18] conducted a study to evaluate the effect of hepatic impairment on the pharmacokinetic properties of liraglutide. Bioavailability of liraglutide appeared to decrease with an increasing degree of hepatic impairment with no significant differences in safety parameters. Clearance of liraglutide is suggested to take place by multiple organ or tissues with no single organ acting as the major route of elimination and hence hepatic impairment is not expected to lead to increased exposure, moreover hepatic impairment in non-diabetic subjects did not increase liraglutide exposure^[19].

Histological changes in liver of obese-diabetic rabbits showed grade III fatty infiltration. Our study showed that liraglutide alone reduced the fatty change from grade III to grade II. However, in combination with glimepiride there was no significant reduction of fatty infiltration in obese-diabetic rabbits.

At present there are limited data available on the effect of liraglutide on hepatic function but our study reveals the influence of liraglutide on hepatic enzymes. Liraglutide did not produce any statistical significant effect on hepatic enzymes particularly AST and ALT, although it has the tendency to decrease these elevated enzymes in obese-diabetic rabbits. Since it's a short term study conducted for 3 weeks, long term studies are essential to establish the effect of liraglutide on hepatic function in obese-diabetics.

5. Conclusion

Liraglutide alone decreased the elevated levels of AST, ALT and reduced the fatty infiltration of liver in obese-diabetic rabbits. However, in combination with Glimepiride it failed to show any improvement in hepatic dysfunction rather worsened the impairment. Hence Liraglutide alone and not in combination with glimepiride is effective in improving the hepatic dysfunction of obese-diabetic rabbits. More studies are required to further determine the clinical use of liraglutide in the management of metabolic syndrome.

6. References

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