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Effect of Liraglutide on Formalin induced tonic pain

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The study was done to find out the effect of Liraglutide on formalin induced tonic pain in obese rabbits. Rabbits were made obese by giving HFD (High fat diet) for 10 weeks. Tonic pain was induced with 5% formalin and it was observed that obese rabbits showed a significantly lesser pain score as compared to normal rabbits. Liraglutide (7 μ g/kg s.c.) was then given to both the group of rabbits for 3 weeks and it was observed that Liraglutide, though produced a significant decrease in body weight, but decrease in tonic pain scores was not significant in obese rabbits.

Keyword: Obesity, Liraglutide, Tonic pain

1. Introduction

Obesity, particularly central (visceral) obesity is the most common metabolic disorder. The prevalence of obesity has been increasing at a rapid rate over the last few decades. The patients with obesity usually have a higher pain sensitivity threshold than people of other categories, so they feel less pain^[1, 2]. Dietary-induced obese rats also were found to be similar to obese humans in being less sensitive to painful stimuli^[3]. In some studies decreased sensitivity to pain was reported in obese human when pain was induced by needle pressure method^[4] or using electrical stimulation method^[5, 6]. In a study, obese rabbits have been demonstrated to be having an increased threshold of formalin-induced tonic pain^[7] Contrary to these findings, other studies have shown more sensitivity to painful stimuli in obese animals and human^[3, 8] Obese Zucker rats were observed to have lower pain threshold^[9] suggestive of a

defect in the endogenous opioid systems. Conflicting observations were also reported in a rat model of obesity for the tail flick latency test- a method that primarily assesses transient phasic pain^[10, 11] Though, obesity is generally accepted to have varied effects on the pain threshold, the mechanism is not known very well. However, obesity has been implicated in alteration of pain by modulating the opioid system in humans^[8] and animals^[3, 9, 10]

Liraglutide, an analogue or mimetic of GLP-1 with a prolonged activity is used for the treatment of type 2 diabetes. It is also effective in reducing body weight and this property of liraglutide is exploited successfully in the treatment of obesity. No study has been done about the responses of liraglutide, an antidiabetic drug on the nociceptive responses in obese subjects. It is reasonable to study the effects of liraglutide on

pain behavior in obese subjects. The present study was carried out to observe the effect of liraglutide on formalin induced tonic pain in obese rabbits.

2. Material and methods

The study was conducted in the Department of Pharmacology in collaboration with the Department of Biochemistry, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Sciences, Rohtak. Approval for the study was obtained from the Institutional Animal Ethical Committee, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Sciences, Rohtak.

2.1 Animals

The study was conducted in New Zealand white adult rabbits weighing approximately 1.5-2 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. Animals were fed with standard rabbit's chow having composition of all dietary elements appropriate for maintaining normal rabbit^[12] After a week of acclimatization period experiments were performed between 9 am to 2 pm to maintain uniformity and avoid diurnal variations. Care was taken to minimize sufferings and pain to the animals. All procedures followed the guidelines of the National Institute of Health, 1996 (Guide for the care and use of laboratory animals).

2.2 Experimental Obesity

To induce obesity, the rabbits were given a 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^[13] Weekly body weight was recorded throughout the period of 10 weeks. Similarly, skin fold thickness (SFT) lateral to the umbilicus, measured in mm was recorded. The rabbits, 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 were considered obese.

2.3 Body Weight and Skin Fold Thickness:

Body weight and skin fold thickness of rabbits were measured before and after pre-treatment, and then weekly for three weeks after inception and at the end of treatment. The body weight was recorded using a standard animal weighing machine and skin fold thickness was measured with the help of vernier calliper.

2.4 Induction of Tonic Pain:

After the animal got adapted to the test environment, 0.1 ml of 5% formalin was injected subcutaneously using a fine needle into the center of planter surface of the paw of right hind limb^[14]. The recording of pain responses in the freely moving animal was immediately started after the injection. Quantification of the pain produced by the formalin injection was done on the basis of observable alteration in the behavior of the animal^[15] Formalin produced biphasic nociceptive responses^[16] The pain intensity was rated according to the standard numerical pain rating scale from 0-3 in decreasing order^[17]

- 0- Normal weight bearing on injected paw
- 1- Limping during locomotion or resting the paw lightly on the floor or normal grooming
- 2- Elevation of injected paw
- 3- Licking or biting the injected paw

Pain was quantified by measuring the amount of time spent by the rabbit in each of four behavioral categories during each time block of 300 sec (5 min) for a period of 60 min^[18] The mean numerical ratings were calculated following the standard procedures^[14, 17].

$$\text{Pain score} = \frac{\sum (\text{respective weight} \times \text{duration(s) in each behavior})}{300}$$

2.5 Experimental Design and Protocol:

The rabbits were divided into two categories as follows-

- A. Normal rabbits
- B. Obese rabbits

Each above category was divided into two study groups having 6 rabbits in each for the purpose of drug treatment as follows:

Group 1. Rabbits received vehicle of inj. Liraglutide daily s.c. for 3 weeks.

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

Rabbits were exposed to formalin-induced tonic pain and the recording of pain responses in the freely moving animal were done immediately after the subcutaneous injection of formalin in the planter surface of right hind paw. Formalin test was performed before and after treatment and weekly during treatment and at the end of the treatment.

2.6 Statistical Analysis: The data of individual rabbits were collected from various study groups and were expressed in terms of mean ±SEM. The statistical significance was assessed by using paired/ unpaired t-test followed wherever appropriate. Intragroup analysis in treatment groups was done using repeated measures analysis of variance (RM-ANOVA) with Bonferroni's correction and intergroup analysis between treatment groups was done using one

way analysis of variance. P value <0.05 was considered significant. All statistical calculations were performed using SPSS software package.

3. Results

3.1 Formalin induced tonic pain scores in obese rabbits

Table 1 shows pain scores measurement of obese rabbits and normal rabbits. Pain scores declined significantly (p<0.05) in obese rabbits compared to normal rabbits.

Table 1: Formalin induced pain scores

Rabbits	Pain Scores (mean±SEM)
Normal (n=6)	1.80±0.06
Obese (n=6)	1.64±0.02*

*Comparison of pain scores of obese rabbits with normal rabbits (p <0.05)

3.2 Effect of Liraglutide on pain scores in obese rabbits:

Table 2 shows the effects of liraglutide (7 µg/kg/day, s.c.) on pain scores in obese rabbits and normal rabbits. There was a further reduction in pain scores in obese rabbits by liraglutide and was highly significant (p<0.01) compared to normal.

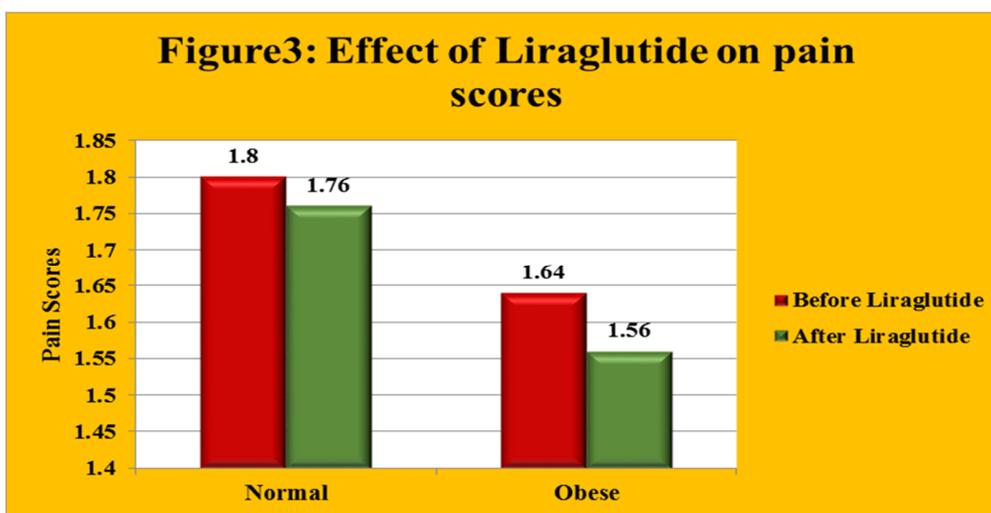
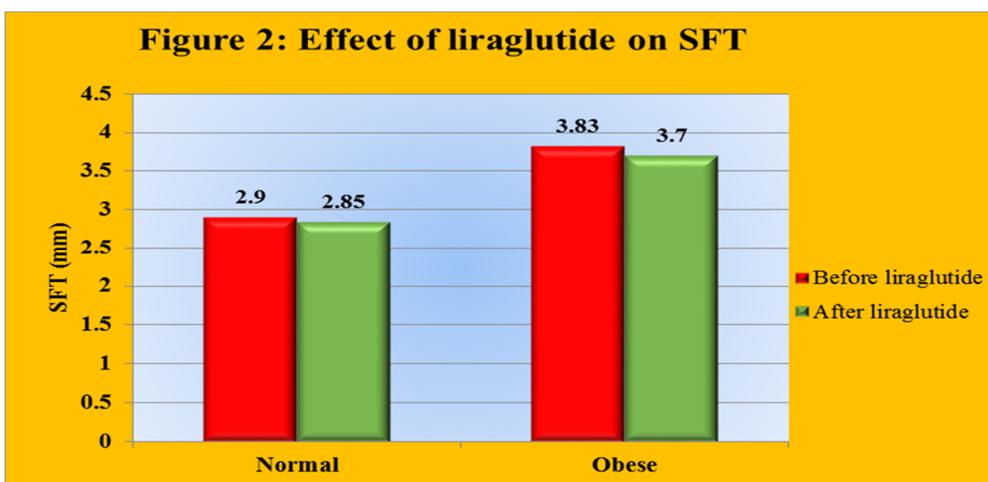
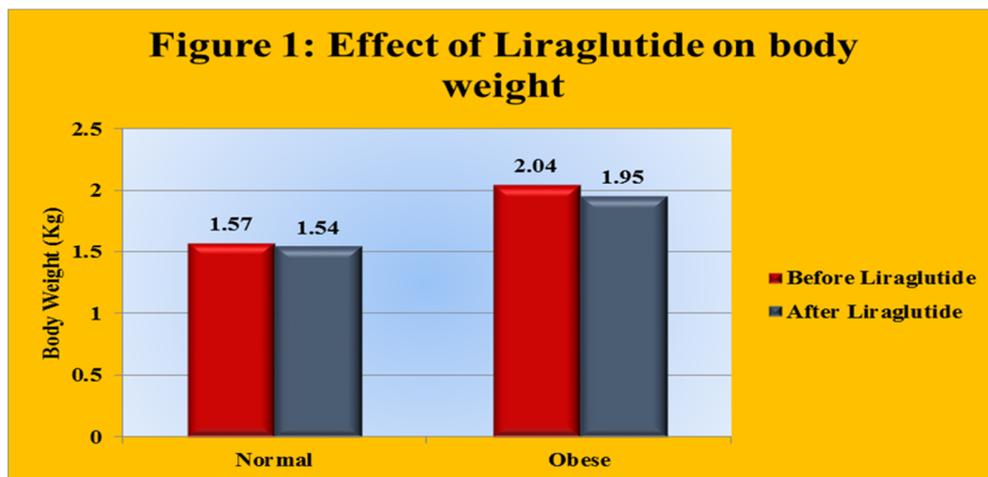
Table 2: Effect of Liraglutide on tonic pain scores.

Rabbits (n=6)	Tonic pain Scores (mean±SEM)	
	Before Liraglutide	After 3 wks liraglutide (7 µg/kg/day, s.c.)
Normal	1.80±0.06	1.76±0.08
Obese	1.64±0.04	1.56±0.02*

*Comparison of tonic pain scores of obese rabbits after liraglutide treatment (p>0.05)

3.3 Effects of liraglutide on tonic pain in relation to body weight and skin fold thickness (SFT) in obese rabbits: Obese rabbits had significantly (p<0.05) high body weight (2.04±0.02 kg) (Figure1) and increased SFT (3.83±0.11 mm) (Figure2) compared to normal

rabbits. Tonic pain was decreased with an increase in the body weight and SFT. Liraglutide, though, decreased body weight (1.95±0.03 kg) and SFT (3.70±0.08 mm) marginally but the changes in tonic pain scores (Figure3) were not significant.



4. Discussion

Tonic pain has been characterized as pain arising from inflammation and has two components. The pain resembles inflammatory pain in human. Liraglutide enhances insulin secretion without causing severe hypoglycemia and does not cause weight gain. A detailed analysis of its pleiotropic or extra glycaemic effects, however, reveals a multifaceted character of the molecule. Liraglutide is effective in reducing body weight, decreasing visceral fat, lowering systolic blood pressure and improving lipid profile as well as other cardiovascular risk markers, while reducing insulin resistance.

In our study, obese rabbits showed a significant high body weight and more skin fold thickness (SFT) as compared to normal rabbits. Liraglutide was found to reduce body weight and SFT in obese rabbits. The reason for reducing body weight and skin fold thickness with liraglutide is probably due to the effects, like delay in gastric emptying, promoting satiety and decreasing food intake. The results of our study were in accordance with the results of many other quoted studies. To the best of our knowledge, no study has been done to demonstrate the status of tonic pain in obese rabbits treated with liraglutide for the purpose of reducing the body weight.

Our study showed lower tonic pain scores in obese rabbits compared to normal rabbits. Obesity has been implicated in the alteration of pain response by modulating the opioid system in human^[8, 19] and animals^[5, 6, 20]. An elevated level of serum β -endorphin has been documented in the obese women exhibiting positive correlation with body weight^[11, 21] and this elevation of serum β -endorphin ameliorates pain perception. Furthermore, another study conducted by Zahorska-Markiewicz B *et al.*^[6] demonstrated that though obesity reduced pain perception due to elevated serum β -endorphin levels, but a weight reducing treatment did not change the pain sensitivity in obese women because altered metabolic responses persist even after reduction

of body weight^[22]. Similarly, liraglutide treatment had shown some reduction in pain scores but not statistically significant which could be explained to the fact that increased serum β -endorphin levels probably persisted even after treatment with liraglutide. It has been established by Basbaum and Field^[23] that increase in β endorphin levels in the cerebrospinal fluids ameliorates pain perception. While, these results were extremely suggestive, but no strong conclusions may be drawn at this point until more studies are carried out to determine the exact mechanism of increase pain threshold in the obesity. Liraglutide given to obese rabbits did not have any significant effect on the pain threshold. Our findings that the tonic pain threshold was increased in obese rabbits and liraglutide failed to affect pain threshold are in agreement with those of Zahorska-Markiewicz B *et al.*^[6]. Obese rabbits showed lesser tonic pain scores as compared to normal rabbits and liraglutide treatment did not alter the tonic pain despite decrease in the body weight.

5. References

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