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## Effects of tetrahydrolipstatin (Orlistat) on basal metabolic rate, biochemical parameters and liver enzymes in rabbits (*Oryctolagus cuniculus*)

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### Abstract

The present study was conducted to determine the effect of orlistat on basal metabolic rate and biochemical parameters and liver enzymes in rabbits during the year 2015. Tetrahydrolipstatin commonly known as orlistat with a chemical formula (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[[(2 S, 3 S)-3-hexyl-4-oxo-2-oxetanyl] methyl]-dodecyl ester, is a lipstatin derivative which is gastric and pancreatic lipase inhibitor. It decreases caloric intake by inhibiting fats digestion and absorption. A 4-wk experiment was conducted using 20 females' rabbits, 6-8yrs of age, to evaluate the effect of tetrahydrolipstatin on cholesterol, weight, glucose, uric acid and liver enzymes of rabbits. The rabbits were randomly distributed in to four groups. A, B, C and D. the grouping were based on the concentration of tetrahydrolipstatin add libitum to female rabbits. Group A was administrated with a standard control diet (control group). Group B, Group C and Group D received 120mg, 240mg and 360mg Tetrahydrolipstatin respectively, experiment duration was 31 days. Weight and cholesterol level was decreased significantly ( $P < 0.01$ ) while, other parameters were also differ significantly at the level of ( $P < 0.05$ ). It has been related to rare cases of acute liver injury.

**Keywords:** Basal metabolic rate, liver enzymes, lipstatin, inhibitor

### 1. Introduction

Obesity has been increased all over the world most probably in developed and developing countries (Hodge and Zimmet, 1994; WHO, 1998; Seidell, 1999) [10, 17, 19]. Obesity especially Abdominal or visceral is closely related to lipid and glucose status disturbance, and also associated with cardiovascular risk (Logue *et al.*, 2011; Goodpaster *et al.*, 2010) [14, 7]. However obesity and overweight are not life threatening but they contribute in many diseases (Równicka-Zubik *et al.*, 2011) [18].

The first strategy for weight loss is a physical activity, combination of diet and behavior modification. But, sometimes in adult patients anti-obesity drugs may be used in combination with diet and life-style modifications have been unsuccessful (Suliburska *et al.*, 2012) [20]. These therapeutics approaches for weight control contain compounds that target both homeostatic and hedonic control mechanisms of the human body (Harrold *et al.*, 2012) [8]. The drug efficiency depends on consumption of freely available food and by reducing the urge to consume food (Jain *et al.*, 2012) [13].

In addition to life style modification anti-obesity pharmacotherapy is considered as an important treatment. The best anti-obesity drug should have following characteristics. It should reduce obesity-related morbidity and modality and cause maintain significant decrease in body weight. In august 1998 the European agency licensed Tetrahydrolipstatin (Orlistat) for the Assessment of Medicinal Products (Foxcroft and Milne, 2000) [6]. It is a gastrointestinal lipase inhibitor. The fat absorption binding to pancreatic lipase is decreased; which block hydrolyses of triglycerides into fatty acids and monoglycerides, therefore increasing faecal fat excretion by 30%. It also causes gastric emptying and secretion of gut peptides (Enç *et al.*, 2009) [5], around ten years have passed that it has been approved for use (Zhi *et al.*, 1994; Hauptman, 2000) [24, 9].

Chemically orlistat is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[[(2 S, 3 S)-3-hexyl-4-oxo-2-oxetanyl] methyl]-dodecyl ester. Its empirical formula is C<sub>29</sub>H<sub>53</sub>NO<sub>5</sub>, and its molecular weight is 495.7 (Isidro and Cordido, 2010) [12].

51% to 82% pancreatic lipase inhibition and 47% to 91% gastric lipase inhibition has been achieved by a lipase inhibitor known as orlistat (Nammi *et al.*, 2004) [15]. About one third of fat absorption is reduced and its minimum absorption has no efficient action (Carrière *et al.*, 2001) [3]. Pharmacokinetic data indicated that orlistat is not significantly absorbed into the systemic circulation in both non-obese and obese persons by using radiolabeled orlistat (Nutley, 1999; Zhi *et al.*, 1995; Zhi *et al.*, 1996) [17, 22, 23, 25]. Total radioactivity of a maximum plasma concentrations occurred in about 8 hours by a single oral doses of C-orlistat that is 50 or 360 mg (Zhi *et al.*, 1995, Zhi *et al.*, 1996) [22, 23, 25]. Orlistat Plasma concentrations were below the detectable assay limit 5 ng/ml at the peak of total radioactivity (Nutley, 1999; Zhi *et al.*, 1995; Zhi *et al.*, 1996) [17, 22, 23, 25].

Patients administrated with 120mg dose of orlistat daily three times a day for 2 years showed no proof of drug accumulation (Zhi *et al.*, 1999) [26]. Though it was believed that all pharmacologic action related to orlistat exerted in gastrointestinal tract and, agents of metabolism were also occur in gastrointestinal wall (Nutley, 1999) [17]. From the studies related to radiolabeled orlistat, its metabolites were recognized. Orlistat contain two major metabolites M1 and M3. M1 has hydrolyzed four member ring of lactone also known as primary metabolite while, M3 is composed up of N formal leucine moiety cleaved with M1 (Zhi *et al.*, 1996) [23, 25].

Open b-lactone ring was present in the chemical structure of both M1 and M3 metabolites of orlistat. For lipase inhibition lactone ring is required due to its low plasma concentrations, and they are supposed to be inactive pharmacologically (Nutley, 1999, Zhi *et al.*, 1999; Zhi *et al.*, 1996) [17, 26, 23, 25].

The present study is aimed to estimate the side effects of Tetrahydrolipstatin and to check the effect of weight reduction with Tetrahydrolipstatin treatment on body metabolic rate, glucose level, uric acid, cholesterol and liver enzymes in rabbits.

## 2. Materials and Methods

Salt (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[[(2 S, 3 S)-3-hexyl-4-oxo-2-oxetanyl] methyl]-dodecyl ester (orlistat) was obtained from Pharmaceutical Industry Islamabad.

This study was carried out on rabbits weighting about 1.30kg to 2.0kg which were used as experimental animals. The rabbits were used in this experiment were females of the species *Oryctolagus cuniculus* which were procured from Veterinary Research Institute Ghazi Road Lahore. The rabbits were kept under observations for about 7 days before the onset of experiment to exclude any intercurrent infection. During the period of research the chosen animal's mere housed in large four partitioned wooden cages at normal temperature (47±37) °C. Each part of the cage contained one group. Rabbits were given standard feed. 6 to 8 years old rabbits were taken and randomly divided into four groups. These groups were labeled as A, B, C and D. Five rabbits were present in each group. Duration of treatment was 4 weeks. Four blood samples were collected with an interval of 7 days. Group A was control group i.e.

given no treatment. Whereas, group B, C and D were supplemented by 120mg, 240mg and 360mg Tetrahydrolipstatin orally respectively.

On each trial day, Blood were collected from the rabbits of all the groups A, B, C and D, with the help of fine needled 5ml syringe from jugular vein and was saved in the gel tubes after 10-12 hours overnight fast. Samples were then labeled and were kept at 4 °C and analyzed within 24 hours.

Weight, glucose, cholesterol, uric acid, and liver enzymes were calculated with an interval of 7 days. Cholesterol was calculated by using Selectra XL2 chemistry auto analyzer. Semi-automatic chemistry analyzer was used to determine the liver enzymes through an end point method at 37 °C by using kits that are available commercially (Iqbal *et al.*, 2013) [11]. Statistically Data was analyzed by SPSS software. The effect of tetrahydrolipstatin on weight, cholesterol, glucose, uric acid and LFT was assessed by two way Anova.

## 3. Results and Discussion

Different parameters have been studied to determine the effect of Tetrahydrolipstatin in rabbits. Table-1 shows the (Mean ±S.E) values of weight, bio-chemical parameters and LFT values in rabbits. There was significant difference on weight loss between control group and experimental group during the experimental work. Similarly, Beermann *et al.* (2001) [2] observed weight reduction of about 2-3% by orlistat during his three months study on Swedish patients. The results of our study are quite in line with findings of above workers. Cholesterol, glucose and uric acid decreased significantly ( $p < 0.01$ ) in all the treated groups. Zavoral, (1998) [21] determined that after the treatment of one year with orlistat weight loss of about 9% was observed. There was also significant reduction of Total cholesterol and low density lipoprotein (LDL) as well as concentration of glucose and fasting insulin was also decreased in patients treated with orlistat. The results of our study are in quite agreement with the findings of above workers. Norris *et al.* (2004) [16] analyzed that non-diabetic patients reduces more weight by using orlistat as compared to diabetic patients. Fasting glucose, glycosylated Hb, total and LDL cholesterol and triglycerides were significantly. Liver enzymes that include bilirubin, Alanine aminotransferases and Alkaline phosphatase also differed significantly in all the treated groups. Douglas *et al.* (2013) [4] observed acute liver injury during immediately before or after the treatment of orlistat. The findings of our study are quite in agreement with the work of above workers. Liver damage before treatment might be due to change in the status of health linked with decision to start treatment rather than the drug effect.

## 4. Summary/Conclusion

From this research it was concluded that Tetrahydrolipstatin is an effective anti-obesity drug in addition to life style modifications for obesity management. But still there is a need to develop new and effective drugs. Tetrahydrolipstatin show significant effect on weight and cholesterol as well as, on other parameters that include glucose, uric acid, Bilirubin, ALP and ALT. The strategies for weight reduction includes: (i) decrease energy intake, by using low calorie diet, (2) enhance energy expenditure. Further studies were required to determine its long term effect and interaction of Tetrahydrolipstatin with other chemical agents.

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