

# The Effect of Venustat on Sex Hormones and Testicular Tissue in Adult Rats

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Received: August 19, 2015

Accepted: November 11, 2015

## ABSTRACT

**Background and Purpose:** Venustat drug is reversible inhibitor of lipase enzyme that inactivates this enzyme by linking with it and prevents triglyceride fat hydrolysis to absorbable form (fatty acids and monoglycerides). Venustat prevents fat digestion. The result of consuming this drug is undigested fat excretion. In this way Venustat reduces calories and weight loss. The aim of this study was to determine the effect of Venustat on pituitary-gonadal axis and spermatogenesis process.

**Methods:** this experimental study was performed on 50 adult male Wistar rats divided into five groups of 10. The control group received no drug treatment the sham group received 1 cc normal saline as a solvent and experimental groups received 50, 100 and 150 milligrams per kilogram of body weight Venustat for 21 days. Blood samples were taken from all the groups and tissue sections were prepared. Serum concentrations were measured by ELISA method. Statistical ANOVA and Tukey tests were analyzed.

**Results:** 100 and 150 milligrams per kilogram of body weight of Venustat reduces the amount of testosterone, dihydrotestosterone and increase LH concentrations and at a dose of 150 milligrams per kilogram of body weight increased FSH concentration ( $P < 0.05$ ).

**Conclusion:** High doses of Venustat decreases serum concentrations of testosterone and dihydrotestosterone, weakens Spermatogenic cell production and increases the concentration of LH and FSH. So probably taking this drug for a long period reduces reproductive activity performance.

**KEYWORDS:** Venustat, testosterone, FSH, LH, rat

## INTRODUCTION

Venustat is produced and distributed under different commercial names including Xenical (the original name of the drug which is made in Germany) and Venustat in Iran. It may not work on half of consumers but when it works on people there will be 5% reduction in BMI<sup>1</sup> within three months of use and 16% of weight loss within 1 year of use (1 and 2).

It is relatively less harmful and its mechanism is different. It has no effect on appetite and prevents the absorption of 1/3 of fat in digestive tract.

In addition, due to the impaired absorption of fat-soluble vitamins, including vitamins A, D, E and K, two hours after Venustat multivitamin supplements should be taken. According to FDA<sup>2</sup> this drug created a risk of breast cancer so the manufacturer withdrew its application for the drug approval. These pills when taken with meals excrete a large percentage of dietary fat (3, 4, 8).

Anti-lipase tablets disrupt fat absorption and thereby impair the absorption of fat-soluble vitamins. In fact, the absorption of vitamins does not happen among the consumers of these tablets. However, if the person uses these tablets for a short period will not have a problem but if they are used for a long term, side effects will occur. These drugs cause the fat-soluble vitamins deficiencies, increased monthly bleeding in women due to a lack of vitamin k in blood (2, 3 and 10). On the other hand, the lack of fat-soluble vitamins like E and has its own complications.

Venustat (Orlistat) is a reversible inhibitor of the lipase enzyme and by linking with this enzyme disables it so it prevents triglycerides hydrolysis into an absorbable fat (fatty acids and monoglycerides). This drug is poorly absorbed orally and linked to the protein at a level of 99%: its half-life is 1-2 hours and 97% is excreted in feces (5 and 12).

When taken with fat-soluble medicines (reducing the absorption of fat-soluble drugs), Venustat has drug interactions with certain medications such as blood thinners (warfarin and aspirin), anticancer drugs such as cyclosporine and some diabetes drugs such as metformin and insulin (6, 11 and 13).

<sup>1</sup> Body Mass Index

<sup>2</sup> Food and Drug Administration

The initial effects of the use of Venustat include:

1. Fatty stool
2. Fecal incontinence
3. Repeated bowel movements
4. Flatulence
5. There is evidence that long-term use of Venustat causes colon cancer (3 and 7).

The main application of Venustat is to reduce obesity. But Venustat has other benefits as well: Venustat reduces the chance of type 2 diabetes by 40%. Long-term use of the drug reduces average blood pressure (4 and 9).

It reduces serum levels of cholesterol, LDL and triglycerides and increases HDL (12).

On the other hand comprehensive reports on the effect of this drug on male reproductive process and the testicular tissue have been performed. For this reason, the effect of Venustat medication on sex hormones and changes in the testicular tissue is analyzed.

## METHOD

This empirical study was conducted in 2014 in the laboratory and 50 Wistar adult male rats weighing 240-250 g were used. At all stages of the research the ethics related to working with laboratory animals based on the Act on care and use of laboratory animals were observed and the related permit was collected from ethics and research committee in Islamic Azad University of Kazerun branch. All animals were kept in in standard lighting conditions 12 hours of light and 12 hours of darkness and in an environment with a temperature of  $23 \pm 2$  degrees Celsius and had access to food and water in sufficient quantities. They were randomly divided into 5 groups of ten in polycarbonate cages based on control, sham and experimental groups. The animals in control group received no drug or non-pharmacological treatment. The sham group received 1 ml of the solvent i.e. distilled water once a day. The experimental groups were fed a daily dose of the Venustat medication with doses of 50, 100 and 150 milligrams per kilogram of body weight for 21 days by feeder syringe. After twenty-one days, the animals weighed, anesthetized with ether and blood samples were taken from their hearts. Approximately 5 ml of blood from each rat was collected in sterile test tubes without anticoagulant substance. The collected blood samples centrifuged for 15 minutes at a speed of 5000 rpm to separate the serum from the clot. Then the samples were kept at  $-20^{\circ} \text{C}$  for measurement of serum concentrations of FSH, LH, testosterone and DHT hormones. Hormone measurement was done by ELISA method. Hormone kits were manufactured by Monibind-CA. After opening the animals' abdomen both testes of all groups were removed and weighed and after preparing tissue sections and staining by hematoxylin-eosin stains by graded lamella for measurement (graticule), changes in Leydig, sertoli cell and spermatogenesis chain cells between the experimental and control groups in studies of tissue were determined by Light microscopy.

The collected data were analyzed by SPSS software ANOVA and Tukey's tests.

## Findings

The results showed that serum concentrations of testosterone and DHT on day 22 had significant reduction in the groups taking 100 and 150 milligrams per kilogram of body weight drug than the control group ( $P < 0.05$ ) Serum concentrations of FSH, LH on day 22 had significant reduction in the groups taking 100 and 150 milligrams per kilogram of body weight drug than the control group ( $P < 0.05$ ) (Table 1).

Histological study of testicular tissue showed that in the group receiving 100 and 150 mg of drug per kilogram, there was a relative decrease in the number of spermatogonia, primary spermatocytes, spermatids and Leydig compared to control and sham groups ( $P < 0.05$ ).

The number of Sertoli cells had no significant difference between the experimental, sham and control groups ( $P < 0.05$ ). Also

There was no significant change in these parameters ( $P < 0.05$ ) (Table 2 and Figs 3-1).

Table 1: The comparison between mean and SD of plasma concentrations of testosterone, DHT, LH and FSH after oral administration of Venustat in different groups

Group	Testosterone (nmol/L)	DHT (ng/dl)	FSH (U/L)	LH (U/L)
Control	5.0 $\pm$ 0.05	46.8 $\pm$ 0.58	0.65 $\pm$ 0.015	1.37 $\pm$ 0.008
Sham	4.9 $\pm$ 0.11	45.7 $\pm$ 0.90	0.65 $\pm$ 0.014	1.40 $\pm$ 0.009
Experimental 1	4.8 $\pm$ 0.11	43.4 $\pm$ 0.92	0.67 $\pm$ 0.018	1.38 $\pm$ 0.007
Experimental 2	4.5 $\pm$ 0.13*	42.5 $\pm$ 0.76*	0.65 $\pm$ 0.017	1.42 $\pm$ 0.008*
Experimental 3	3.6 $\pm$ 0.16*	32.55 $\pm$ 1.25*	0.71 $\pm$ 0.009*	1.53 $\pm$ 0.015*

\* Significant difference with the control and placebo groups ( $P < 0.05$ )

Table 2: The comparison between mean and SD of sperm lineage, Sertoli and Leydig cells in Seminiferous tubules after oral administration of Venustat in different groups

Group	Number of spermatogonia cells	The number of spermatocytes cells	No. Spermatid cells	Sertoli cells	Leydig cells
Control	88.8 ± 3.36	102.4 ± 4.50	204.4 ± 4.51	28.2 ± 2.96	14.9 ± 0.95
Sham	87.4 ± 3.33	101.8 ± 3.94	200 ± 3.77	27.8 ± 2.38	13.3 ± 0.47
Experimental 1	81 ± 3.26	98 ± 3.98	191 ± 4.59	25 ± 2.61	11.2 ± 0.59
Experimental 2	79 ± 3.46	92.5 ± 3.65	182.5 ± 4.77*	24.5 ± 2.87	11.6 ± 0.73*
Experimental 3	67.8 ± 2.23*	75.5 ± 3.47*	179.4 ± 5.23*	22.2 ± 2.38	102 ± 0.66*

\* Significant difference with the control and placebo groups (P<0.05)

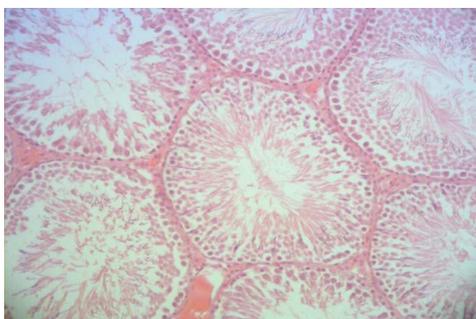


Figure 1: testicular tissue photo micrograph in the control group (H & E staining, optical microscope, magnification x10)

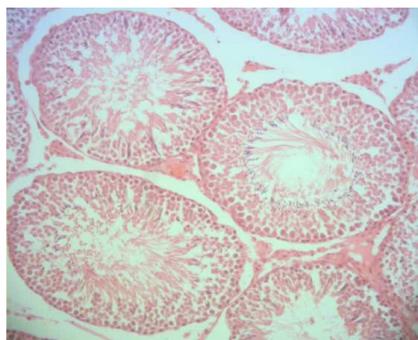


Figure 2: testicular tissue photo micrograph in experimental group 2 at a dose of 100 milligrams per kilogram (H & E staining, optical microscope, magnification x10)

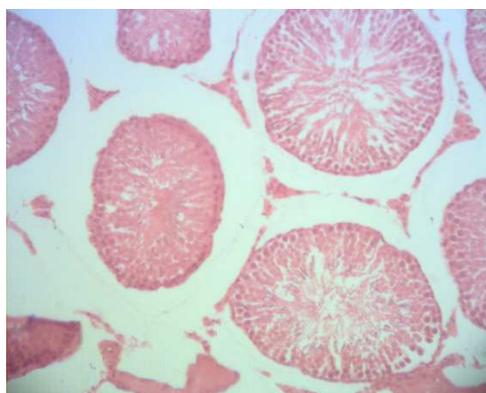


Figure 3: testicular tissue photo micrograph in 3 experimental groups at a dose of 150 mg per kilogram (H & E staining, optical microscope, magnification x10)

## DISCUSSION

The purpose of this study was to evaluate the effect of Venustat on sex hormones (FSH, testosterone and DHT hormones) and changes in testicular tissue in adult male rats.

The results of this study showed that oral administration of Venustat reduces testosterone and DHT hormones and increases LH and FSH significantly and testicular tissue studies revealed a significant reduction in seminiferous cell chain. Studies show that Venustat releases serotonin and cortisol (14). An increase in this neurotransmitter associated with cortisol inhibits the activity of 17-hydroxylase enzyme and 17-20 Desmolase by increasing the enzyme 11 beta hydroxysteroid and by affecting the number of Leydig cells impairs the function of steroids' production. Inhibiting the activity of enzymes involved in the production of testicular steroids reduces testosterone and DHT (15 and 16). As a result of the increase in neurotransmitter, ACTH<sup>3</sup> is reduced indirectly (17). So with ACTH reduction the adrenal cortex cells' activity to produce steroids is reduced and the most important phase to induce ACTH to regulate the secretion of the adrenal cortex i.e. the activation of protein kinase A to convert cholesterol to pregnenolone is weakened. With respect to the reduction in testosterone through the negative feedback, the secretion of FSH, LH from the anterior pituitary increases (18).

The results of this study in decreased levels of testosterone and DHT confirm the studies conducted by other researchers on Venustat through non-induction effect of liver enzymes and its effect on the reduced metabolism of estrogen hormone and its increased plasma levels that reduces the level of testosterone and DHT (19). Studies of other researchers show that there is a neural pathway between the brain and the testes the induction of which by corticotropin releasing factor (CRF) affects the Leydig cells' function (20).

Testosterone is the inhibitor of monoamine oxidase enzyme that is involved in the catabolism of dopamine and the reduction of this enzyme increases the amount of dopamine (21). So probably by reducing testosterone, this inhibitory effect on the activity of this enzyme is reduced and the concentration is reduced as well. Dopamine by affecting arcuate nucleus inhibits FSH hormone production and dopamine reduction increases gonadotropins (22).

Previous studies have shown that testosterone affect the Sertoli cells directly. Sertoli cells feed dividing sex cells by tubular liquid secretion. It also secretes different proteins such as growth factor, transferrin and bioactive peptides such as Prodynorphin and nutrients each one playing a special role in sex cells division. Testosterone also has another role which is the direct effect dividing sex cells (23, 24). With regard to the role of testosterone on spermatogenesis and also its production in Leydig cells, it is clear that reducing the number of Leydig cells as a result of increased estrogen activity caused by Venustat treatment, has reduced testosterone, sexual cells and sperm density (19, 25).

### Conclusion

In general it can be concluded that one of the side effects of Venustat medication is the reduced steroid genesis in testicular tissue. Venustat decreases serum concentrations of testosterone, DHT and increased LH, FSH and impaired reproductive activity. Therefore it should be applied cautiously in patients with impaired production of sex hormones. Also it is suggested to use it along with drugs activating steroid production to reduce side effects in these patients.

### Acknowledgement

Hereby the efforts and contributions of officials and personnel of Azad University of Kazerun, Faculty of Science and clinical laboratory of Dr. Ghavami- Shiraz are appreciated.

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<sup>3</sup> Adrenocorticotrophic hormone

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