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Histological Effect of Orlistat on the Testes in the Male Rats *Rattus Rattus*

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Abstract
Obesity is one of the common diseases of the era, which spread strongly in our life. The development of science led to the find out of many Drugs, the purpose of which was to find the optimal treatment to get rid of excess weight for humans, and through scientific follow-ups, it was seen that anti-obesity treatments (which are many types) It has many side effects, and in most cases, it appears suddenly without calculating the effect. In this research, the anti-obesity drug Orlistat was studied, done on 25 rats in which obesity was induced by feeding them high-fat Diet(HFD), where the rats were divided into five groups (10=negative control group in30 and 60 days),(10 positive control group were feeding HFD only in 30 and 60 days 60 days),(10 = were oral administration with orlistat 50 mg / kg /day in30 and 60 days),(10= were oral administration with orlistat 100 mg /kg /day in 30 and 60 days),(10= were oral administration with orlistat 150 mg /kg /day in period30 and 60 days).studied the initial body weight and final body weight, also in this present study examine the relative organ weight(testes) then examined the histological and physiological effect on tests as an important organ for production. Results revealed significant decrease in final body weight and relative weight of tests in treated rats with orlistat compared with negative control and (HFD) group, histological study show pathological change were observed and this is increased with increase in concentration of orlistat, the conc. 150 mg /kg /day was more efficient as compared with negative control group and positive control group (HFD).

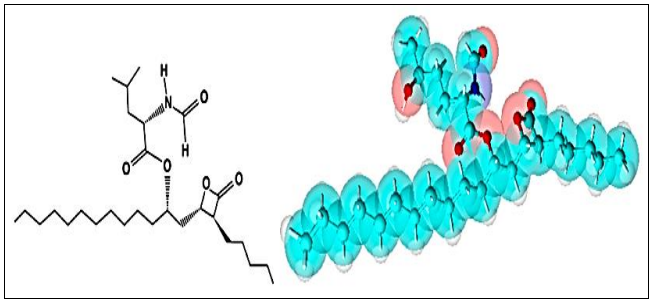
Keywords: Orlistat, Obesity, Testes, Necrosis

Introduction
Orlistat is used as an anti-obesity, where it is a pancreatic lipase inhibitor, it is used as a drug in many countries (Shirai, K., *et al*., 2019) [1].
This drug belongs to the family of lipstatin, it comes from a gram-positive bacterium, Streptomyces toxytricini (Kitadokoro, K *et al*, 2020) [2].
The name of orlistat in the IUPAC is (2S)-1-[(2S,3S)-3-hexyl-4-oxooxetan-2-yl] tridecan-2-y(2S)-4-methyl2-(2-xoethyl) pentanoate, the Y-shaped chemical structure of it is divided into three parts: long β-tetradecanyl alkyl chain, amino ester moiety (N-formyl- L-leucine substituent extending of the C5 bbcarbon atom), and α-heptyl alkyl tail. It is currently accessible as an anti-obesity medication on the market (Xenical or Alli). Which acts locally to inhibit gastric lipases, which are required for long-chain triglyceride digestion (Kitadokoro, *et al*; 2020) [2].
There are no specific structural details on how orlistat molecules bind with this lipase. Orlistat (tetrahydrolipstin) is a Food and Drug Administration (FDA) approved weight-loss medication (Kassab *et al*., 2020) [3]. According to the WHO, the global estimation of overweight people was around 1.9 billion, among that 650 million were obese (World Health Organization, 2011). Obesity increases the possibility of having chronic conditions like hypertension, dyslipidemia, type 2 diabetes, osteoarthritis, stroke, gall stones, and other cardiovascular disorders. Being thinner reduces the risk of illness and death associated with specific medical conditions (Priyadharshini, 2019).
Orlistat binds covalently to the active site on pancreatic lipase and forms a stable complex (Nguyen, *et al*. 2020) [14]. The complex induces a conformational change in the enzyme that leads to a lid-like structure on the lipase, hence exposing the catalytic active site. This operation leads to acylation of a hydroxyl group on serine residue burden on the active site of the enzyme making it inactive as lipase. The inactivated lipase is unable to hydrolyses fats into fatty acids and monoglycerides, which lead to their passage with faces (Al-Omar, *et al*; 2006) [11]. It is hypothesized that the adverse and unpleasant side effects associated with inhibition of digestive enzymes, such as diarrhea, abdominal pain and bloating, as well as the counterproductive effect on appetite, can drive patients to increase energy consumption or sporadically discontinue their course of treatment (Joyce, P; *et al*; 2019). Major side effects of orlistat are gastrointestinal side effects such as flatus.

Obesity

Obesity is defined as a body mass index (BMI) of ≥30 kg/m2, and severe obesity may be defined as a BMI ≥40 kg/m2 or ≥35 kg/ m2 together with comorbidities. Weight reduction was shown to decrease morbidities associated with obesity. Weight loss may also provide a reduction in mortality associated with obesity. By weight reducing interventions. In general, The World Health Organization (WHO) defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk to health [Chooi, Y. C., Ding, C., & Magkos, F. (2019] [5].

For adults, current guidelines from the US Centers for Disease Control and Prevention (CDC) and the WHO define a normal BMI range as 18.5 to 24.9, whereas a BMI ≥25 kg/m2 is considered to be overweight, and a BMI ≥30 kg/m2 is classified as obese, with severe obesity defined as a BMI ≥40 kg/m2, lifestyle intervention may provide 5-7% weight loss, but maintenance of weight loss is difficult. Medical management in obesity aims to reduce weight by 5-10 %. In clinical studies, with medical therapy, weight loss of 4-8 % is typical [Topaloglu, O., & Sahin, I. (2021] [4].



Source: Candela, M. F., 2021

Fig 1: Orlistat structure (left) and optimized geometry (right)

Materials and Methods

Experimental Animals:

The present study will achieve on males white rat Rattus rattus (fifty) were used in this experiment selects rats most aged more than eight weeks and weights ranging from (250-350) g were obtained from the animals house in Faculty of Science, University of Kufa. They should be in good health. The rats are placed in plastic cages with metal covers, 48 cm length, 15 cm wide and 7 cm height. The sawdust, which should be replaced three times a week, is considered in its care to clean the hatching of the special diet and plastic bottles can be used to make a watering tough with a cork equipped with metal pipes. The animals are placed under suitable laboratory conditions in terms of temperature 18-26 Cº and light/dark cycle 10/14 and ventilation rate time/hour 10-15 and also the relative humidity 30-70 (Tan & Tan, 2017).

Drug used:

To prepare the doses of the orlistat used in this study were dissolved in water for the purpose of preparing different doses. The compound of the orlistat was used in this experiment in the form of a capsule from a Hikma pharmaceutical company, Jordan.

Experiment Groups:

The experiment involves the use of 50 male albino rat divided into fifth groups:

1. The first group (Negative Control group): including 10 male rats treated with water and normal food. which sacrifice after 30 and 60 days
2. The second group (Positive Control group): including 10 male rats feeding with HFD which sacrifice after 30 and 60 days.
3. The third group (First treated group): including 10 male rats feeding with HFD+oral administration with orlistat 50/mg/kg/day which sacrifice30, 60 days after treatment.
4. The fourth group (Second treated group): Including 10 male rats feeding with HFD+ oral administration with orlistat 100/mg/kg/day which sacrifice in 30, 60 days after treatment.
5. The fifth group (third treated group): including 10 male rats feeding with HFD+ oral administration with orlistat 150/mg/kg/day which sacrifice 30, 60 days after treatment.

Weight Calculations:

Calculate of Percentage of Body Weight Gain

Percentage of body weight gain (%) = final body weight-initial body weight /initial body weight*****100

Calculate of Organ Relative Weight

Relative organ weight (g/100g bw) =Absolute organ weight / final body weight*****100 (Lackner *et al*., 2019) [7].

Sacrifice and Histological Preparation:

Animals will sacrifice after end of experiment by combination of ketamine:Xylazine (90mg/kg:10mg/kg intra peritoneal), used ketamine 0.5ml and xylazine 0.1ml to each 250g of body weight for anesthesia the animals for all groups, after the anesthesia the animal put in anatomical dish and made linear incision by scissors in abdominal region for collection the blood and liver, by anatomical tools. The adipose tissues were removed were placed on a filter paper to be weighed with sensitive balance. Saved in containers contain 10% formalin (AlTameemi, 2014) [10]. for histological preparation.

Histological Preparations:

All samples fixed after remove them from animals in containers contains 10% formalin (38%100ml formalin in 900ml tap water) and then done series of processed in series steps (Survarna *et al*., 2018) [9]:

1. Dehydration and Clearing

Done withdrawal water from the tissue by a series of incremental concentrations of ethanol (70%, 80%, 90%, 100%, 100%) for two hours in each concentration then in to xylene for ten minutes.

2. Infiltration

After the completion process, the samples transferred into glass containers contains a mixture of paraffin wax with a melting point 57-60 Cº and filter and xylene in the rat of 1:1 for half an hour inside oven in order to keep the wax stable and to ensure impregnation then transferred into paraffin wax inside oven for two hours then moved again to glass containers contains paraffin wax inside oven for two hours.

3. Embedding

Templates were created of wax container samples where wax is poured into special iron molds, the models were

buried and left at laboratory temperature to solidify and then separated from the mold and preserved until the time of cutting.

4. Sectioning
The Rotary Microtome was used for sectioning the patterns and your thickness 5 micrometer, then the ribbons floated at water bath (45-50 Cº) for a minute for it to flatten and after that the sections was put in a clean slides and allowed to dry on hot plate to dry at a temperature 37 Cº.

5. Staining and Mounting
Used the following special dyes for tissue differentiation:

Harris Hematoxylin: A general base color used to stain the nucleus in dark blue:

Eosin Stain: A general acid color used to stain the cytoplasm in red color,

Microscopic Examination:
Compound light microscope was used to study the histological changes in liver, kidneys and testes. Photos were taken to visualize some of results using a light microscope supplied with Optika camera**.**

Statistical Analysis:
All values are expressed as mean ± standard error of mean. Differences between initial and final of animals body weight in each group were analyzed by the paired *t* test. Comparison of body weight, percentage of body weight gain and relative weight of organs between experimental groups was analyzed through 2-ways analysis of variance followed by the Duncan test. Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 23.0).

Results and Discussion
Results: Body Weight
Effect of Interactions of Oral administration of Different Concentrations of Orlistat on Both Final Body Weight (g) and Percentage of Weight Gain (%) in Male Rats:
The results revealed a significant decrease ($p \leq 0.05$) in final body weight of male rat after Oral administration with 50, 100 and 150 mg/kg/day of orlistat compared with both negative and HDF control groups as the following ((291.700±7.083, 270.800±4.268,244.800±1.737, 311.000±5.800, 243.000± 3.265) g respectively compared with the initial body weight as the following (244.1000± 0.674,245.500± 0.806,245.900± 0.8875,244.2000±0.891 and 216.600±0.979)g respectively, and the results showed the concentration 150 mg/kg/day of orlistat caused a more significant decrease ($p<0.05$) in final body weight of male rat compared with the concentrations 50 and 100 mg/kg/day of orlistat and also with both negative and positive(HFD) control groups respectively (Table 1).

Table 1: Effect of interaction of Oral administration of different concentration of orlistat 50, 100and 150 mg/kg/day on final body weight (g) and percentage of weight gain (%) in male rats

Orlistat mg/kg/week	Initial body weight(g) (Mean± S.E)	Final body weight(g) (Mean± S.E)	Weight gain (%) (Mean± S.E)	Gain(+) or loss (-)
Negative Control	216.600±0.979 (B,b)	243.000± 3.265(D,a)	12.194±1.469 (C)	+
Positive Control(HDF)	244.2000± 0.891 (A,b)	311.000±5.800 (A,a)	27.321±2.113 (A)	+
50+HDF	244.1000±0.674 (A,b)	291.700±7.083(B,a)	19.504±2.897(B)	+
100+HDF	245.500±0.806(A,b)	270.800±4.268 (C,a)	10.320±1.803 (D)	+
150+HDF	245.900±0.8875 (A,b)	244.800±1.737 (D,a)	2.397±0.458 (E)	+

*The different letters (Capital letters for column and small letters for row) refers to significant differences ($P < 0.05$) between means while similar letters refers to non-significant differences between means.

The results exhibited a significant decrease ($p \leq 0.05$) in percentage of weight gain of male rat after oral administration with 50, 100 and 150 mg/kg/day of orlistat (19.504±2.897, 10.320±1.803, 2.397±0.458)% respectively compared with both negative and positive (HDF) control groups (12.194±1.469 and 27.321±2.113) % (Table 1).

Effect of Interactions of Oral administration Periods of Orlistat on Both Final Body Weight (g) and Percentage of Weight Gain (%) in Male Rats
The results showed significant decrease ($p<0.05$) in final body weight of male rats after 30 and 60 days of of Oral administration with orlistat (259.600±4.401, 284.920± 6.543) g respectively as compared to initial body weight (238.760±2.388, 239.760 ± 2.358) g respectively (Table 2).
The results revealed significant increase ($p<0.05$) in percentage of weight gain after 30 and 60 days of oral administration with orlistat (9.437±1.373, 19.257±2.203) (%) respectively and the period 60 days of oral administration caused more significant increase ($p<0.05$) compared with 30 days (Table 2).

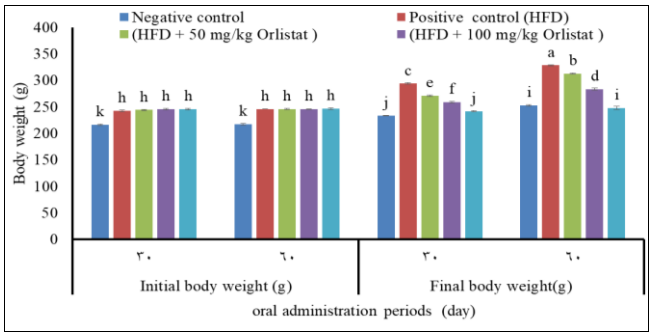
Table 2: Effect of interaction of different oral administration periods of orlistat on body weight and percentage of weight gain (%) in male rats

Days	Initial body weight(g) (Mean± S.E)	Final body weight(g) (Mean± S.E)	Weight gain (%) (Mean± S.E)	Gain(+) or loss (-)
30	238.760±2.388 (A,b)	259.600±4.401 (B,a)	9.437±1.373 (B)	+
60	239.760± 2.358(A,b)	284.920± 6.543(A,a)	19.257±2.203 (A)	+

*The different letters (Capital letters for column and small letters for row) refers to significant differences ($P < 0.05$) between means while similar letters refers to non-significant differences between means

Interactions Between Different Concentrations and Periods of oral administration of Orlistat on Both Final Body Weight (g) and Percentage of Weight Gain (%) in Male Rats

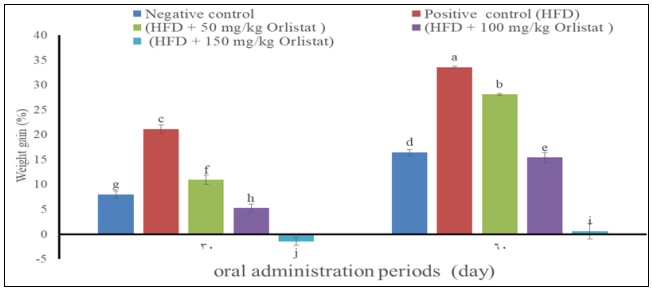
The results shows significant decrease ($p \leq 0.05$) in final body weight compare to initial body weight and also show significant decrease ($p \leq 0.05$)in the final body weight for male rats that treated with concentrations 50,100 and 150 mg/kg/day of orlistat compared to both negative and positive control group in each periods and among periods (Fig 2).



*The different letters refers to significant differences ($P \leq 0.05$) between means while similar letters refers to non-significant differences between means.

Fig 2: Interaction between different concentrations and periods of oral administration of orlistat on body weight (g) in male rats

The results revealed significant decrease ($p \leq 0.05$) in percentage of weight gain between different concentration and periods of oral administration of orlistat compare to both negative and positive(HFD) control group in each periods and among periods (Fig 3).



*The different letters refers to significant differences ($P \leq 0.05$) between means while similar letters refers to non-significant differences between means

Fig 3: Interaction between different concentrations and periods of oral administration of orlistat on weight gain (g) in male rats

Organ Weight:

Effect of Interactions of Oral Administration of Different Concentrations of Orlistat on Testes Relative Weight (g/100g of Body Weight) in Male Rats

The results showed a significant decrease ($P \leq 0.05$) in the relative weights of testes(0.379± 0.019, 0.373± 0.020, 0.340± 0.014) g/ 100g after oral administration with 50, 100 and 150mg/kg/day of orlistat compared to positive control groups(HFD) (0.484±0.011) whereas no significant decrease with negative control (0.233±0.011).

Table 3: Effect of interaction of oral administration of different concentration of orlistat on relative weight of testes (g/100g) of body weight) in male rat

Orlistat mg/kg/day	Relative organ weight g/100g)
	Testes (Mean± S.E)
Negative ontrol	0.233±0.011
Positive Control	0.484±0.011
50+HDF	0.379± 0.019
100+HDF	0.373± 0.020
150+HDF	0.340± 0.014

*The different letters in each column refers to significant differences ($P \leq 0.05$) between means while similar letters refers to non-significant differences between means

Effect of Interactions of Different Oral Administration Periods of Orlistat on Testes Relative Weight (g/100g of Body Weight) in Male Rats:

The results revealed significant decrease ($P \leq 0.05$) in relative weight of testes (0.353±0.019 and 0.343±0.019) g/100g of body weight after 30 and 60 days of oral administration with 50, 100 and 150 mg/kg/day of orlistat and the period 60 days of oral administration caused more significant decrease ($P \leq 0.05$) compared with 30 days (Table 4).

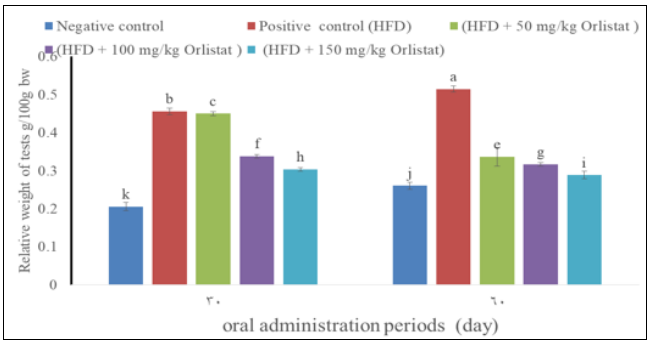
Table 4: Effect of interactions of different oral administration periods of orlistat testes relative weight (g/100g of body weight) in male rats

Days	Testes (mean ± S.E)
30	0.353±0.019 (A)
60	0.343±0.019 (B)

*The different letters in each column refers to significant differences ($P \leq 0.05$) between means while similar letters refers to non-significant differences between means

Interactions Between Different Concentrations and Periods of oral Administration of Orlistat on Testes Relative Weight (g/100g of Body Weight) in Male Rats

A significant decrease ($P \leq 0.05$) showed in relative weight of testes 30- and 60-days periods of oral administration of concentrations 50,100 and 150 mg/kg/day of orlistat compared with both negative and positive control groups and between the periods of oral administration and concentrations (Fig 4).



*The different letters refers to significant differences ($P \leq 0.05$) between means while similar letters refers to non-significant differences between means

Fig 4: Interaction between different concentrations and period of oral administration of orlistat on relative weight of testes g/100g bw in male rats

Histological Studies:

The testes: The histological sections of the testis treated with orlistat at a concentration of 50, 100 and 150 mg / kg / day showed an effect that ranged from medium to severe, as the intensity of stimulation increased with increasing time and concentration, as the number of spermatozoa appeared to be low and completely disappeared by increasing the concentration to 150 and 100 for a period of 60 days, and it showed the presence of calcification in the cells, also giant cells, increased in interstitial apace.

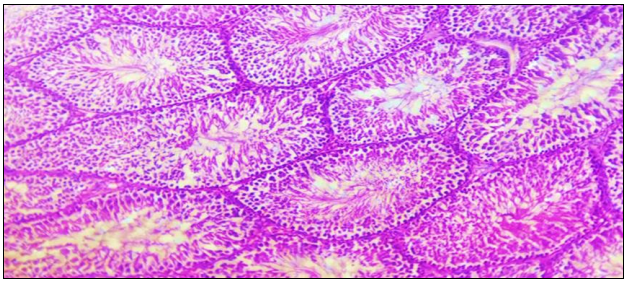


Fig 5: Cross section of the testes treated with distilled water (negative control group) show normal histological architecture of seminiferous tubule (H&E stain, 10x)

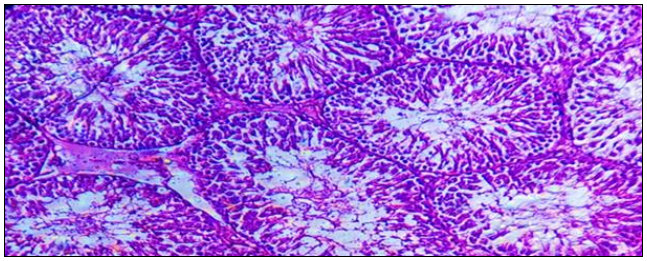


Fig 6: Cross section of the testes treated with High Fat Diet (positive control group) show normal histological architecture of seminiferous tubule (H&E stain, 10x)

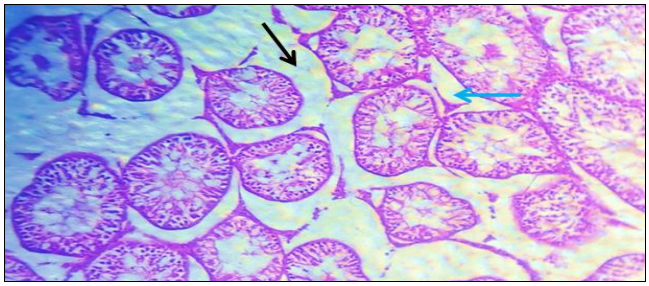


Fig 7: Cross section of the testes treated with 50 mg/kg/day for 30 days shows few spermatid with increase in interstitial space (black arrow). decrease number of lydic cell (blue arrow) (H&E stain, 10x)

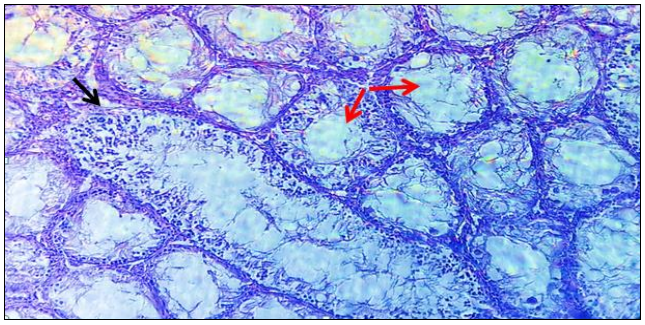


Fig 8: Cross section of the testes treated with 50 mg/kg/day for 60 days hows. (H&E stain, 10x).showed reduction in number of spermatocyte (red arroew) giantisim in cell (black arrow)

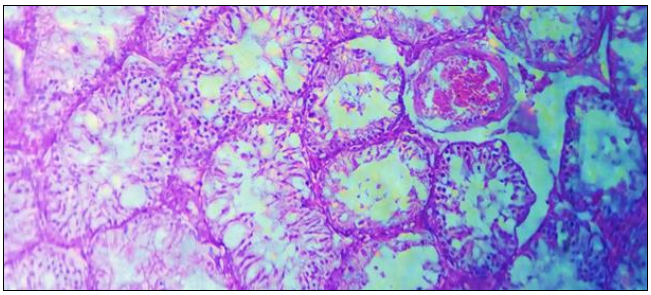


Fig 9: Cross section of the testes treated with 100 mg/kg/day for 30 days. Show necrosis in testicular cells with calcification (red arrow) (H&E stain, 10x)

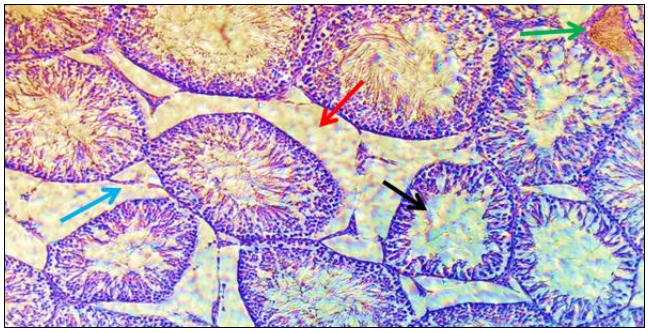


Fig 10: Cross section of the testes treated with 100 mg/kg/day for 60 days shows increased in interstitial space (red arrow) congestion (green arrow), reduction in number of spermatocyte (black arrow), destruction in lydeic cell (blue arrow). (H&E stain, 10x)

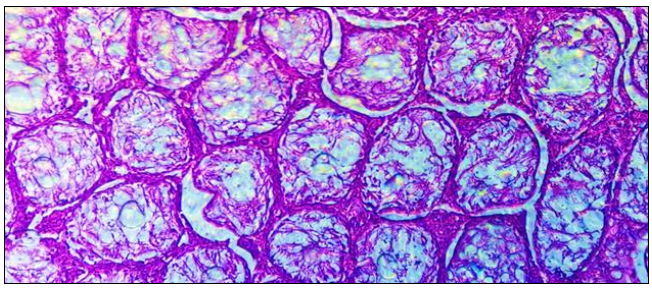


Fig 11: Cross section of the testes treated with 150 mg/kg/day for 30 days shows no sprtmatid with necrosis with inflammatory cells. (H&E stain, 10x)

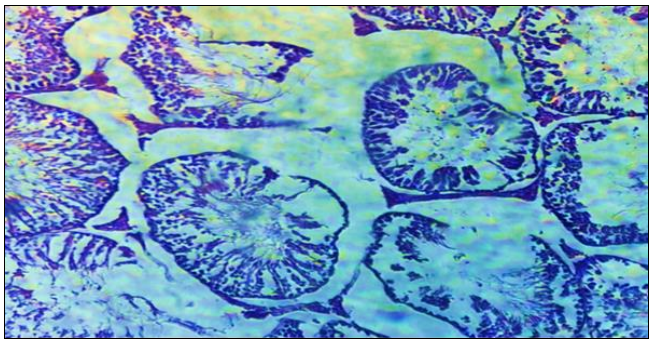


Fig 12: Cross section of the testes treated with 150 mg/kg/day for 60days shows destruction in seminiferous tubule, no sperm, necrosis (H&E stain, 100x)

Conclusions

Treatment with orlistat 50, 100, 150 mg/kg/day caused testes injurey and although orlistat's capacity to reduce weight in obese by inhibiting gastric and pancreatic lipase.but long term treatment cause testes injury with reduced number of spermatocyte.

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