Histological and physiological effect of Orlistat on The liver in The Male Rats *Rattus rattus*

Dhamya Kadhim Sarhan* and Ali Hassan Abood
Department of Biology, Faculty of science, University of Kufa, Iraq
***Corresponding author E-mail: dhamyak.eleiwi@uokufa.edu.iq**

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 50 rats in which obesity was induced by feeding them high-fat Diet(HFD), where the rats were divided into five groups of 10 rats per group(10=negative control group in 30 and 60 days),(10 positive control group were feeding HFD only in two periods 30 &60 days),(10= were oral administration with orlistat 50 mg /kg /day in two period 30 &60 days),(10= were oral administration with orlistat 100 mg /kg /day in two period 30 &60 days),(10= were oral administration with orlistat 150 mg /kg /day in two period 30 &60 days).stuydied the initial body weight and final body weight,also in this present study examine the relative organ weight (liver) then examined the histological and physiological effect on liver as an important part of the body. Results revealed significant decrease in final body weight and relative weight of liver in treated rats with orlistat compared with negative control and (HFD)group, significant increase in Liver enzyme ,pathological change in the liver tissue necrosis and congestion of blood vessel, degeneration in hepatocyte ,results displayed that 60 days were more effect than 30 days and conc.150 mg/kg/day was more effected .

Key worlds: Orlistat, Obesity, Liver ,Biochemical parameter, Liver

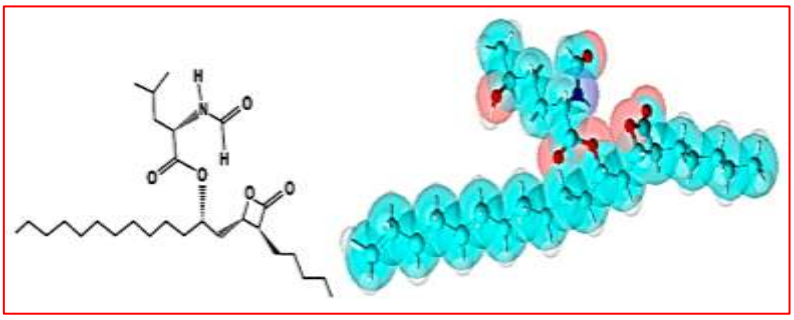
INTRODUCTION

Orlistat is an inhibitor of pancreatic lipase and is used as an anti-obesity drug in many countries (Shirai, *et al*; 2019). Orlistat belongs to the lipstatin family,
derived from the gram-positive bacterium *Streptomyces toxytricini* (Kitadokoro, *et al*., 2020). The IUPAC name of orlistat is (2S)-1-[(2S,3S)-3-hexyl-4-oxooxetan-2-yl] tridecan-2-y(2S)-4-methyl2-(2-xoethyl) pentanoate Its Y-shaped chemical structure is divided into three fragments: amino ester moiety (N-formyl- L-leucine substituent extending of the C5 bbcarbon atom), α-heptyl alkyl tail, and long β-tetradecanyl alkyl chain (Fig. 1). Orlistat is a potent inhibitor of human gastric and pancreatic lipases that play an important role in dietary fat digestion(Li, X.*etal*.,2022) It is currently available in the market as an anti-obesity drug (Xenical or Alli), which acts locally to block gastric lipases that are crucial for the digestion of long-chain triglycerides(Kitadokoro, K *etal*;2020). However, there is no detailed structural information on how orlistat molecules bind to these lipases. Orlistat (tetrahydrolipstin) is a weight-loss marketed drug approved by the Food and Drug Administration (FDA). It has been proved as a highly selective inhibitor of gastric and pancreatic lipases enzymes whose main function is digesting dietary fat. In the digestive tract, orlistat can bind to the lipase enzyme preventing hydrolysis of triglycerides into free fatty acids, which are absorbable for the cells and the undigested triglycerides are then excreted in feces(Joyce,*etal* ., 2020). Thus the

inhibition of lipases by orlistat will lead to reduced caloric intake. At present, orlistat is used for clinical treatment of obesity related type 2 diabetes and cardiovascular diseases. Orlistat may have other clinical applications as in the treatment of chylous ascites which mostly is associated with cirrhosis (Kassab, 2020). Orlistat binds covalently to the active site on pancreatic lipase and forms a stable complex(Nguyen, *etal* .,2020). 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). 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, intestinal cramps, borborygmus, fecal incontinence, oily spotting. The side effects may be observed in up to 30% of the patients (Topaloglu, and Sahin, 2021 ; Holmbäck, *etal*., 2020).

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]. 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].



(Figure 1). Orlistat structure (left) and optimized geometry (right)
(Candela, M. F., 2021).

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, Jorden. according to the groups mentioned in the design of the study.

Experiment Groups

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

a-The first group (Negative Control group): including 10 male rats treated with water and normal food. This group subdivided into two subgroup including 5male rats which sacrifice in 30 and 60 days

b-The second group (Positive Control group): including 10 male rats feeding with HFD This group subdivided into two subgroup including 5male rats which sacrifice in 30 and 60 days.

c- The third group (First treated group): including 10 male rats feeding with HFD+oral administration with orlistat 50/mg/kg/day This group subdivided into two subgroup including 5male rats which sacrifice in 30 and 60 days after treatment.

d- The fourth group (Second treated group): including 10 male rats feeding with HFD+oral administration with orlistat 100/mg/kg/day This group subdivided into two subgroup including 5male rats which sacrifice in 30 and 60 days after treatment.

e- The fifth group (third treated group): including 10 male rats feeding with HFD+oral administration with orlistat 150/mg/kg/day This group subdivided into two subgroup including 5male rats which sacrifice in 30 and 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).

Blood Collection and Serum Sampling

Animals weight has been recorded before and after the dosage by using an electrical balance , Blood samples (2.0–5.0 ml) were obtained (immediately after sacrificing) by heart puncture and put into a gel tubes and permitted to clot . Serum was separated by centrifugation for 15 minutes at 3000 rpm. The isolated serum was kept at -20°C until the estimation of the investigation(Baltazar-Gaytan *et al.*,2019).

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).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) **:**

A) 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.

B) 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.

C) 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.

D) 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.

E) 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 ($p<0.05$) 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 ($p<0.05$) 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<0.05) in final body weight compare to initial body weight and also show significant decrease (p<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 (figure1)

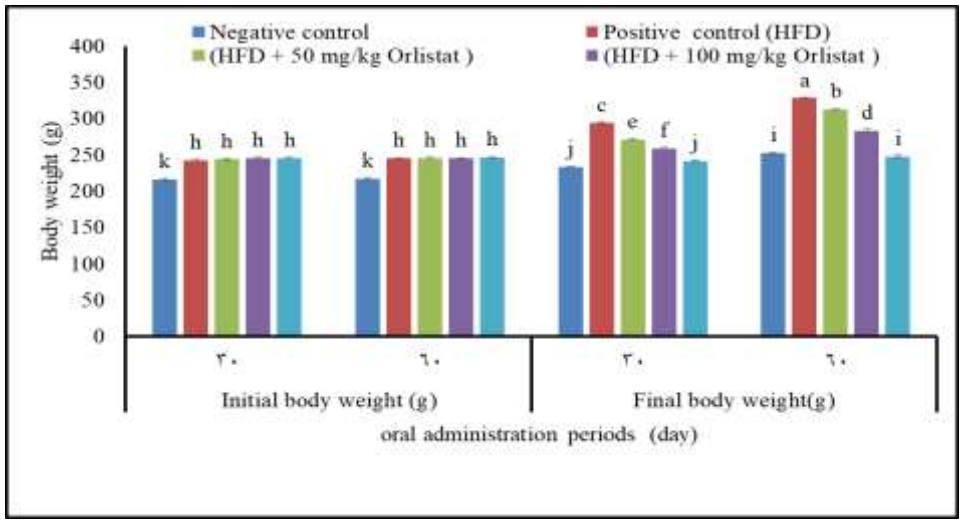


Figure (1): Interaction between different concentrations and periods of oral administration of orlistat on body weight (g) in male rats.
*The different letters refers to significant differences (P< 0.05) between means while similar letters refers to non-significant differences between means.

The results revealed significant decrease (p<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 (figure 2).

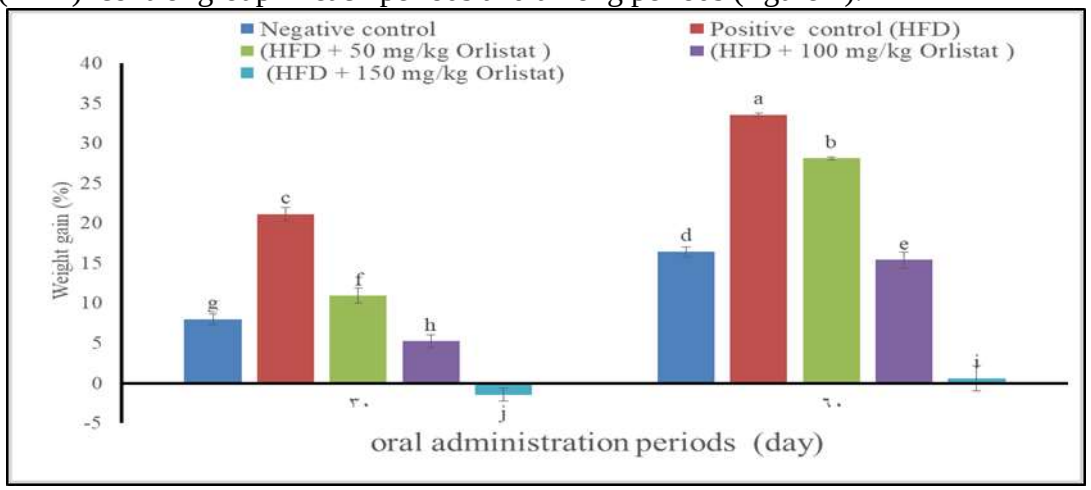


Figure (2): Interaction between different concentrations and periods of oral administration of orlistat on weight gain (g) in male rats.
*The different letters refers to significant differences (P< 0.05) between means while similar letters refers to non-significant differences between means

Organs Weight

Effect of Interactions of oral administration of Different Concentrations of orlistat on Liver Relative Weight (g/100g of Body Weight) in Male Rats.

The results showed a significant decrease ($p<0.05$) in the relative weights of liver (3.750±0.047, 3.572±0.054, 3.015±0.124) g/100g after oral administration with 50, 100 and 150mg/kg/day of orlistat compared to negative and positive(HFD) control groups (3.235±0.113, 3.872 ±0.030) g/100g and(0.233±0.011, 0.484±0.011) g/100g of body weight respectively**.**

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

Orlistat mg/kg/day	Relative organs weight g/100g)
	Liver (Mean± S.E)
Negative ontrol	3.235±0.113 (B)
Positive Control	3.872 ±0.030 (A)
50+HDF	3.750±0.047 (C)
100+HDF	3.572±0.054 (D)
150+HDF	3.015±0.124 (E)

*The different letters in each column refers to significant differences (P< 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 Liver Relative Weight (g/100g of Body Weight) in Male Rats:

The results revealed significant decrease ($p<0.05$) in relative weight of liver (3.693±0.044, 3.285±0.091) 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 ($p<0.05$) compared with 30 days (table 4).

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

Days	Relative organs weight (%) L Liver (Mean± S.E)
30	3.693±0.044 (A)
60	3.285±0.091 (B)

*The different letters in each column 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 Liver Relative Weight (g/100g of Body Weight) in Male Rats:

A significant decrease ($p<0.05$) showed in relative weight of liver for 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 (figure 3).

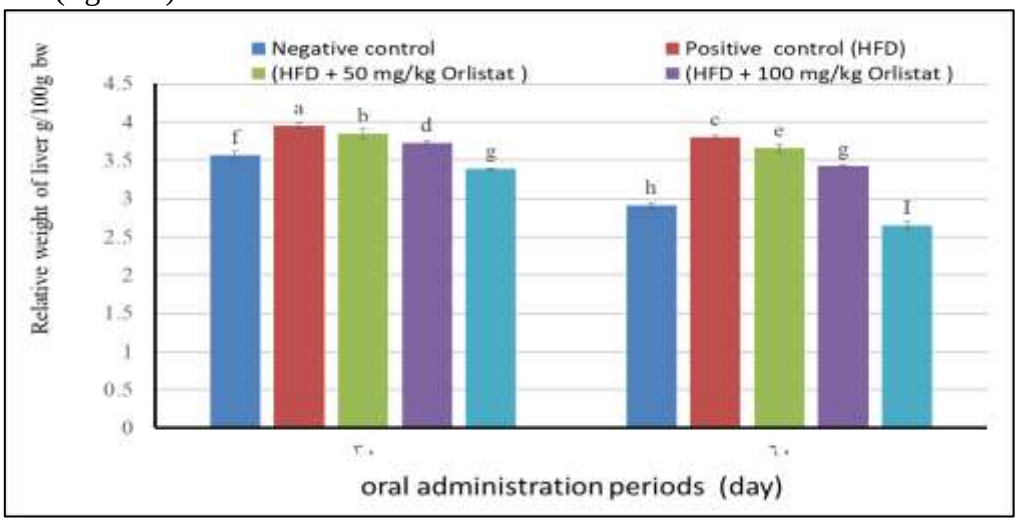


Figure (3): Interaction between different concentrations and period of oral administration of orlistat on relative weight of liver g/100g in male rats.

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

Biochemical parameters

Liver function

Effect of Interactions of oral administration of Different Concentrations of orlistat on ALT, ALP, AST (IU/L) , and Total Bilirubin (mg/dl) in Male Rats:

The results revealed a significant increase ($p<0.05$) in ALT after oral administration with 50,100 and150 mg/kg/day of orlistat (46.200±1.019, 54.800±0.940,73.700±2.695) IU/L respectively compared with both negative and positive (HFD) control groups(29.000±1.316, 40.000± 1.201) IU/L respectively and the results showed the concentration 150 mg/kg/day of orlistat caused a more significant increase ($p<0.05$) compared with the concentrations 50 and100 (mg / kg / day of orlistat (table7). The results exhibited a significant increase ($p<0.05$) in AST after oral administration with 50, 100 and 150 mg/kg/day of orlistat (186.900±3.328, 211.600±7.479,and 236.460±7.930) IU/L respectively compared with both negative and positive control(HFD) groups (29.000±1.316 and 169.520±1.933) IU/L respectively and the results showed the concentration 150 mg/kg/day of orlistat caused a more significant increase ($p<0.05$) compared with the concentrations 50 and 100 mg/kg/day of orlistat (table 7). The results revealed a significant increase ($p<0.05$) in ALP after oral administration with 50, 100 and 150 mg/kg/day of orlistat (162.900±2.203, 174.300±2.944, and 193.300±6.969) (IU/L) respectively compared with both negative and positive(HFD) control groups (95.200±1.171,and 144.600±0.858) (IU/L) respectively and the results showed the concentration 150 mg/kg/day of orlistat caused a more significant increase ($p<0.05$) compared with the concentrations 50 and 100 mg/kg/day of orlistat (table7). The results revealed asignificant decrease (($p<0.05$) in total serum bilirubin T.S.B after oral administration with 50,100,150 mg/kg/day of orlistat (1.973±0.054,1.409±0.082, and 1.171±0.088) (mg/dl) respectively compared with both negative and positive control groups (1.752±0.009 ,and 2.303±0.072) (mg/dl) respectively and results showed the concentration 150 mg/kg/day of orlistat caused more significant decrease ($p<0.05$) compared with the concentration 50 and 100
mg/kg/day of orlistat(table 7).

(Table 7): Effect of interactions of different oral administration of different concentration of orlistat on liver enzyme in male rats.

Orlistat mg/kg/day	ALT (IU/L) (Mean± S.E)	AST (IU/L) (Mean± S.E)	ALP(IU/L) (Mean± S.E)	T.S.B(mg/dl) (Mean± S.E)
Negative Control	29.000±1.316 (E)	78.890±0.735 (E)	95.200±1.171 (E)	1.752±0.009 (C)
Positive Control(HFD)	40.000±1.201 (D)	169.520±1.933 (D)	144.600±0.858 (D)	2.303±0.072(A)
50+ HFD	46.200±1.019 (C)	186.900±3.328 (C)	162.900±2.203 (C)	1.973±0.054 (B)
100+ HFD	54.800±0.940 (B)	211.600±7.479 (B)	174.300±2.944 (B)	1.409±0.082 (D)
150+ HFD	73.700±2.695 (A)	236.460±7.930 (A)	193.300±6.969 (A)	1.171±0.088 (E)

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

Effect of Interactions of oral administration Periods of orlistat on ALT, AST ,and ALP(IU/L) and Total Bilirubin (mg/dl) in Male Rats:

The results showed significant increase ($p<0.05$) in ALT(45.600±2.735 and51.880±3.524) (IU/L),AST (164.604±9.519and188.744±12.618) IU/L and, ALP (147.240±5.853and160.880±8.032),while results showed significant decrease in total bilirubin(1.750±0.047and1.692±0.120) mg/dl respectively 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 increase ($p<0.05$) in ALT,AST.ALP compared with 30 days,results showed period 60 day of oral administration caused more significant decrease (table 8).

Table (8): Effect of interactions of different oral administration periods of orlistat on ALT, AST,ALP (IU/L) and total bilirubin (mg/dl) in male rats.

Days	ALT (IU/L) (Mean± S.E)	AST (IU/L) (Mean± S.E)	ALP(IU/L) (Mean± S.E)	T.S.B (mg/dl) (Mean± S.E)
30	45.600±2.735 (B)	164.604±9.519(B)	147.240±5.853 (B)	1.750±0.047(A)
60	51.880±3.524 (A)	188.744±12.618(A)	160.880±8.032 (A)	1.692±0.120(B)

*The different letters in each column 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 0f Orlistat on ALT, AST,ALP (IU/L) and Total Bilirubin (mg/dl) in Male Rats:

A significant increase ($p<0.05$) showed in ALT, AST,ALP and total bilirubin for 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 ,and significant decrease in total bilirubin for 30 and 60 days periods of oral administration of concentrations 50,100,150 mg/kg/day of orlistat compared with both negative and

positive control groups and between the periods of oral administration and concentrations (figure 6, 7, 8,and 9) respectively**.**

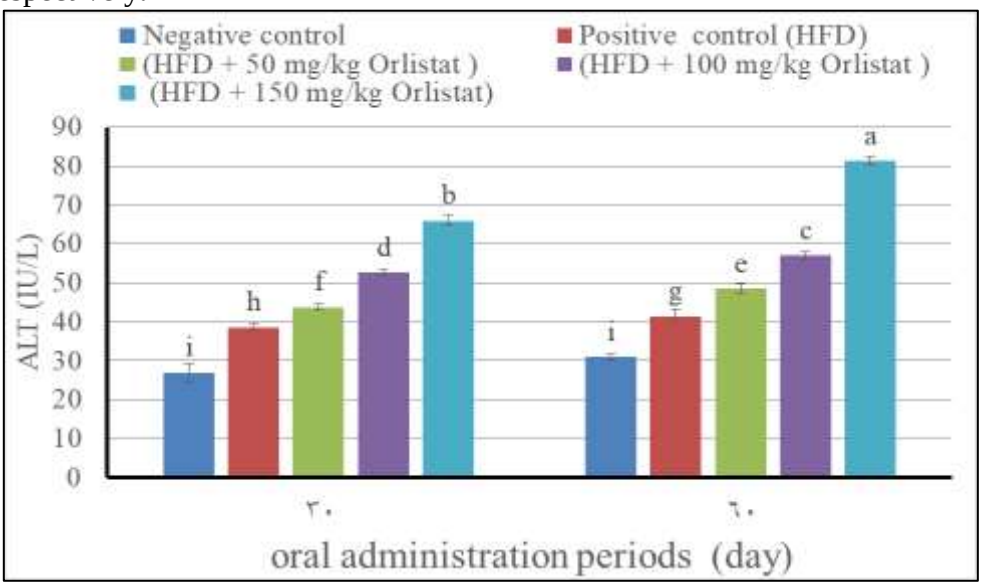


Figure (6): Interaction between different concentrations and period of oral administration of orlistat on ALT (IU/L) in male rats.

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

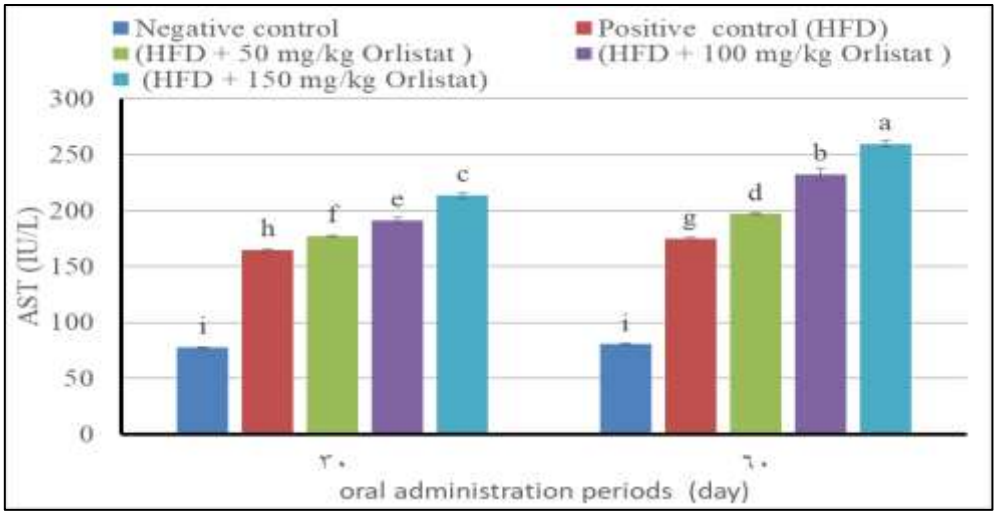


Figure (7): Interaction between different concentrations and period of oral administration of orlistat on AST (IU/L) in male rats.

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

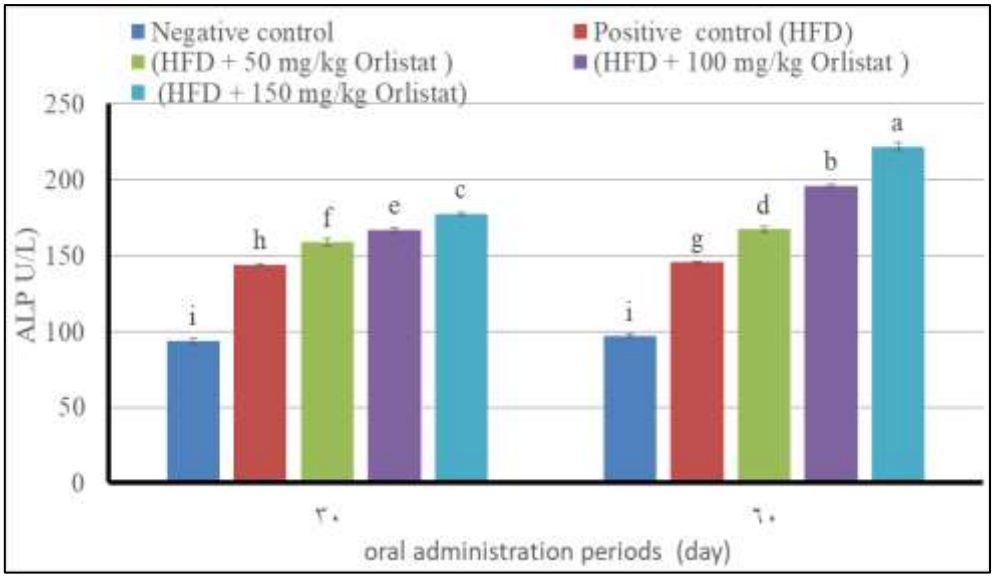


Figure (8): Interaction between different concentrations and period of oral administration of orlistat on ALP (mg/dl) in male rats.

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

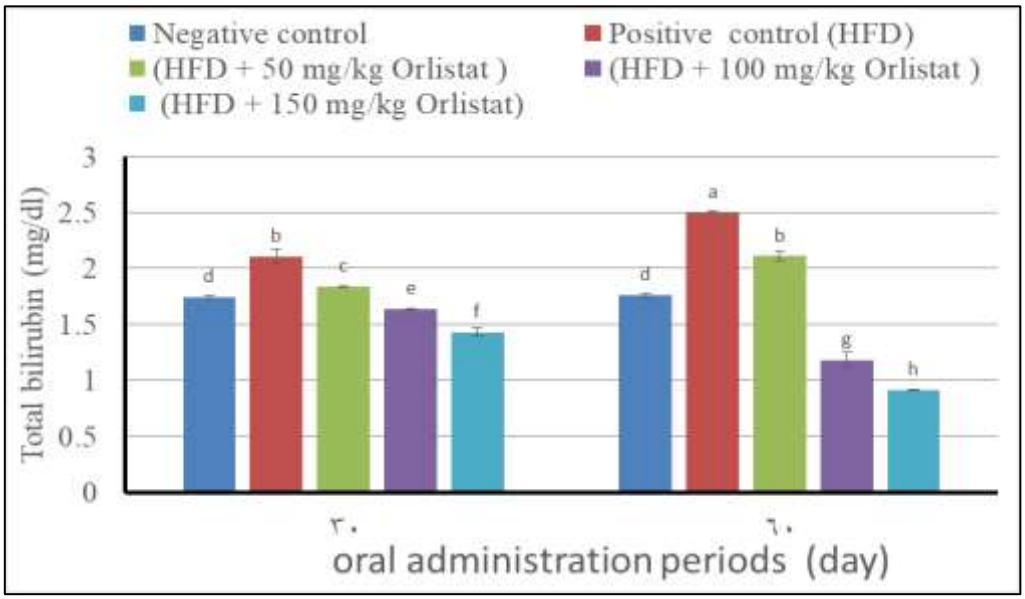


Figure (9): Interaction between different concentrations and period of oral administration of orlistat on TSB (mg/dl) in male rats**.**

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

Histological studies

Liver study

The histopathological study of males rats liver revealed mild to severe changes in liver histology at the dose of 50 mg/kg/day for 30 days of orlistat illustrated mild necrosis, congestion (figure 10). 60 days all lesions observed at 30 days continous, in addition to more sever hepatic blood vessels congestion (figure 10).

Rat treated with 100,150 mg/kg/days, for 30 days also showed same changes but more sever. 60 days figure showed sever congestion of blood vessels and diffuse vacuolar degeneration in hepatocytes while 60days shows multi focal inflammatory necrosis, degeneration in hepatocyte

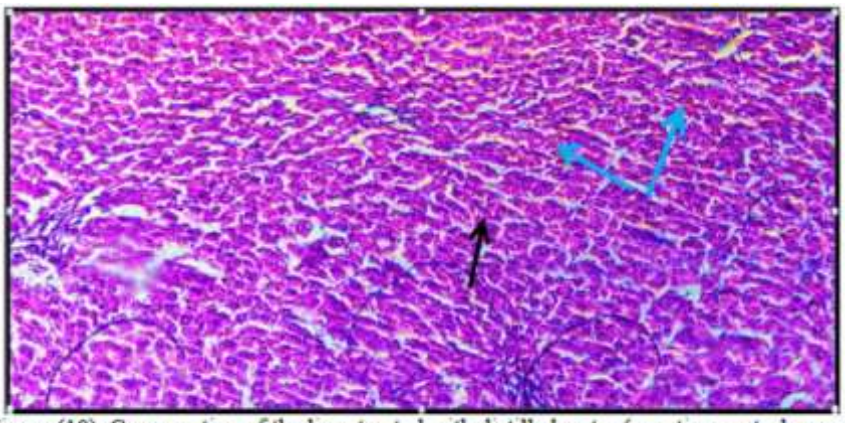


Figure (10): Cross section of the liver treated with distilled water (negative control group) show hepatocyte (➔)sinosoide (⟶) H&E, 10x.

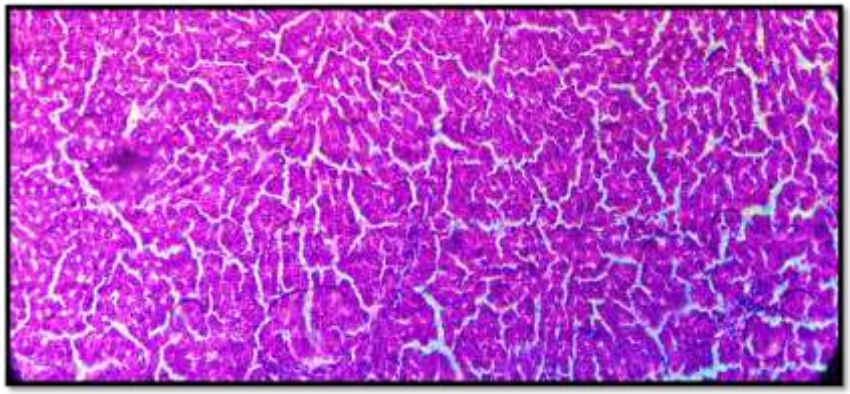


Figure (11): Cross section of the liver feeding with high fat diet(HFD) (positive control group) show hepatocyte

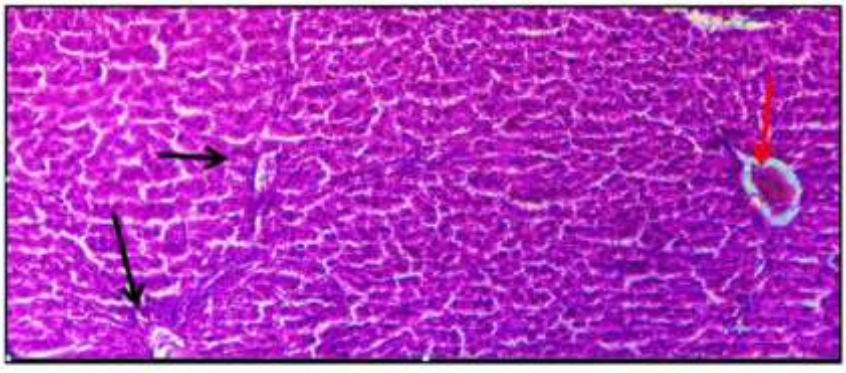


Figure (4- 15): Cross section of the liver treated with 50 mg/kg/day for 30 days shows degeneration in hepatocyte(black arrow),congestion of blood vessele . (red arrow) (H&E stain, 10x).

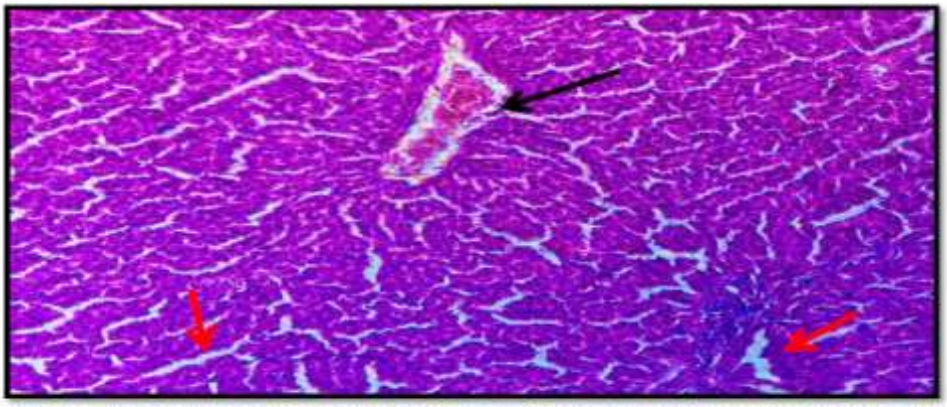


Figure (4- 16): Cross section of the liver treated with 50 mg/kg/day for 60 days shows congestion in central vein ( ) degeneration in hepatocyte ( )(H&E stain, 10x).

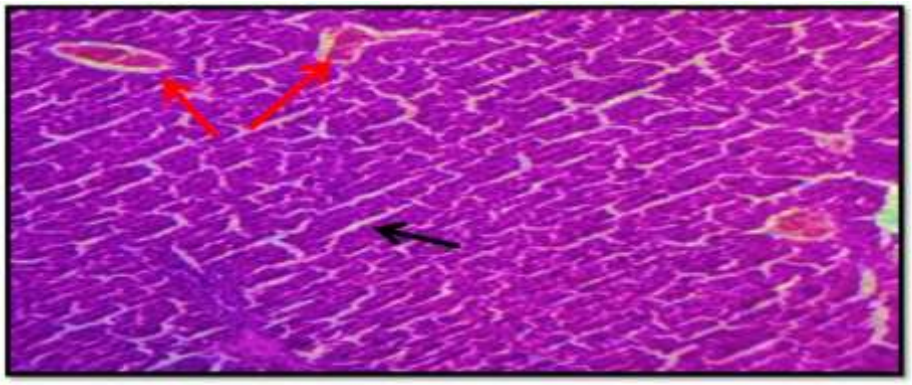
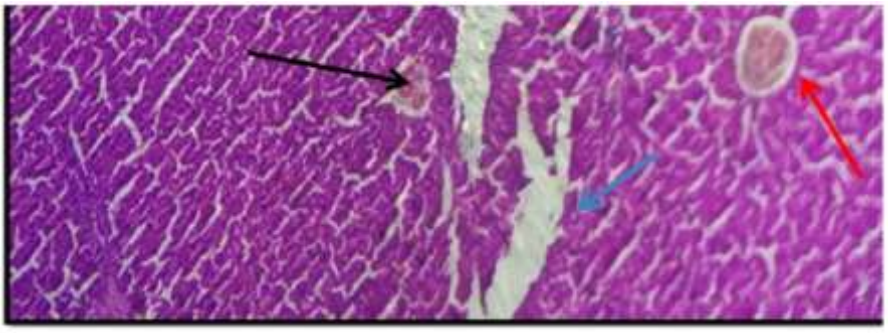


Figure (4- 17): Cross section of the liver treated with 100 mg/kg/day for 30 days shows congestion in central vein ( ) degeneration in hepatocyte ( ) . (H&E stain, 10x).



Cross section of the liver treated with 100 mg/kg/day for 60 days shows congestion in central vein( ) degeneration in hepatocyte ( ) . pyremia ( ) (H&E stain, 10x).

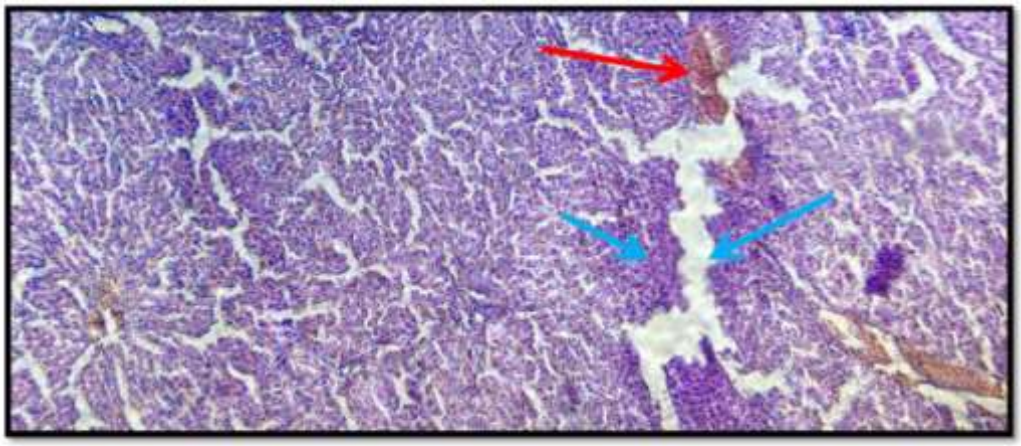


Figure (4- 19): Cross section of the liver treated with 150 mg/kg/day for 30 days shows congestion ( ), necrosis and aggregation of inflammatory cells ( ) (H&E stain, 10.)

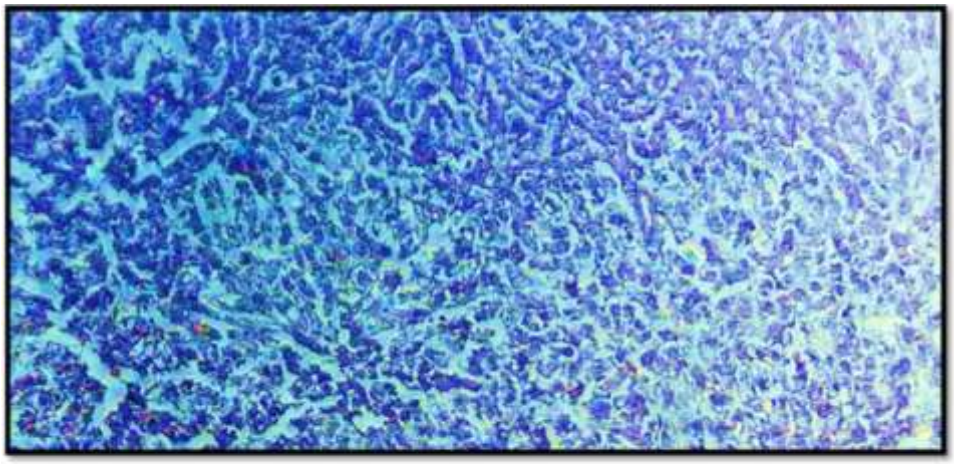


Figure (4- 20): Cross section of the liver treated with 150 mg/kg/days for 60 days shows degeneration in hepatic cell,multifocal inflammatory cell and necrosis(H&E stain, 100x)

DISCUSSION

The present study reveals significant decrease ($p<0.05$) in final body weight of male rats after oral administration with 50, 100, and 150 mg/kg/day of orlistat as compared to the initial body weight, whereas there was significant decrease ($p<0.05$) in both final body weight and percentage of weight gain as compared to negative and positive control groups after oral administration with 50, 100, and 150 mg/kg/day of orlistat. In our previous study we demonstrated that oral administration of orlistat concomitantly with HFD for 30 &60 days in body weight compared to the initial weight when treating rats with orlistat, and this is due to the mechanism action of orlistat, which is concerned with inhibiting the pancreatic lipase and gastric lipase ,this enzyme is responsible for the breakdown of triglycerides and fatty acids. HFD group gained weight more than the NC group, with a significantly higher BMI, reflecting an increase in body fat and not increase in growth, as demonstrated by a higher Lee obesity index. Our findings corroborate previous studies in human (Morales et al., 2016) and animals (Karimi et al., 2015; Samat et al., 2017) in which orlistat reduced both energy intake and body weight**.** This result corresponds with several studies that showed orlistat has a weight-loss effect by improving metabolism through its influence on fat absorption, where orlistat is covalently bonded to the active site in Pancreatic lipase and forms a stable complex This combination causes the

enzyme to alter conformation, resulting in a cap-like structure on the lipase(Li S,etal; 2020, Nguyen PT.,etal;2020), our present study in compliance with results ((Karimi, etal; 2015).

a significant decrease in relative body weight level in treated obese rats with Orlistat when compared with obesity groups. (Tousson, E., etal; 2018). , significant reductions in liver fat content were observed with orlistat treatment for 24weeks compared with routine treatment (Ye, J., etal.,2019).and this results agreed with this present study. . The adipose tissues secrete high levels of adipokines and free fatty acids into the portal vein, which subsequently result in the increased concentration of free fatty acids in the liver tissue.(Paschos,etal., 2004).

Bio chemical parameters

Liver enzyme

The results in present study revealed that serum level of was significantly ($P<0.05$) elevate in all experimental groups compared to negative and positive control and this finding agrees with previous studies which found that the abuse of high doses and long period of exposure of orlistat result in increases the level of liver enzyme . Orlistat is generally well tolerated and was approved by the Food and Drug Administration in 1998 and its safety has been supported by a comprehensive body of data (Mirja *et al*., 2004). However, severe liver injury and hepatic failure has been reported in controlled clinical trials in which patients treated with orlistat discontinued treatment due to adverse events (Heck *et al*., 2000).

In our present study revealed significant decrease($P<0.05$) in bilirubin concentration in male rats which oral administrated with various doses of orlistat compared with negative and positive control.This results agreed with previous studies , (Hafkamp *et al*., 2003). hypothesized that orlistat treatment decreases plasma bilirubin concentration in rats by increasing turnover and fecal excretion of bilirubin, Bilirubin is a waste product made from old blood cells. Tests for bilirubin levels help to determine if the liver is functioning appropriately .

CONCLUSIONS

Treatment with orlistat 50 ,100,150 mg /kg/ day caused liver injurey and elevation in liver enzyme ,although orlistat's capacity to reduce weight in obese by inhipiting gastric and pancreatic lipase.but long term treatment cause liver injurey .

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