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Histological and Physiological evaluation of Hydroxycut and Refit orlistat hepatotoxicity in male albino rats

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Abstract

Obesity has become common all over the world, threatening people's daily lives and also threatening animal health as well as increasing the risk of obesity-related diseases. Dietary supplements such as Hydroxycut and Refit orlistat targeting obesity have gained popularity and are now a prevalent cause of drug-induced liver impairment and hepatotoxicity. This study aims to assess the effect of Hydroxycut and Revit Orlistat on the liver histologically and physiologically. Male albino rats were employed in the current investigation, which ran from August 29, 2022, until April 29, 2023. Thirty blood samples from albino male rats were used in the investigation. Six groups of five rats each were created from the categorization of the rats. One of the groups served as control group. The results of histological examination of liver of rats showed various changes, ranging from the appearance of desquamation of the central vein wall, hemorrhage, vacuolation of hepatocyte, dilation of sinusoids, Kupffer cells, and necrosis as well. This study quantified the concentrations of AST and ALT in blood serum and demonstrated a noteworthy elevation in both. Furthermore, there were statistically significant disparities at a probability threshold of less than 0.05. The study demonstrated that Hydroxycut and Orlistat exert a direct detrimental impact on liver tissue, leading to the development of acute liver failure. Therefore, it is important to consider the toxicity of these drugs while prescribing them, especially in elderly patients and patients with kidney and liver disease.

Introduction:

Orlistat is an effective, irreversible lipase enzyme inhibitor (ORL) [1]. It is used in concert with a low-calorie, low-fat diet to treat obesity, including weight loss and control. A person's health may be at risk due to obesity, Obesity is characterized by an abnormal accumulation of adipose tissue in the body. The global prevalence of obesity has been observed to increase over time, and Malaysia is one country where approximately 13.1% of individuals are classified as obese [2]. Health professionals are increasingly concerned about the growing prevalence of obesity due to its association with serious health conditions such as diabetes mellitus, metabolic sickness, coronary heart disease, hypertension, some types of cancer, and other obesity-related disorders [3].

Nutritional supplement usage is prevalent in the United States (US), however it remains mainly unregulated. Obesity has emerged as a significant public health issue in the United States. Recent research indicates that the obesity rate among adults exceeds 30%, while the percentage of overweight individuals stands at 65%. [4].

Orlistat (ORL) plays a role in the lessening the possibility of gaining weight following weight loss. Patients who are obese and have an initial BMI of 30 kg/m2 or more, or who have additional risk factors (such as diabetes, hyperlipidemia, or hypertension), may be prescribed it [5]. Because of its poor solubility over physiological pH ranges and slow rate of dissolution, ORL has a low therapeutic efficacy when used either locally or routinely. This classification places ORL in BCS class II medicine. Consequently, it is necessary to administer higher doses, which may raise the probability of experiencing severe adverse effects [4]. At present, almost 40% of recently formed chemical compounds derived from efforts in medicine development are lipophilic or have low solubility in water.

One of the main causes of liver damage is drug usage. There have been reports of over 900 medications, poisons, and plants causing liver damage; of these, drugs are responsible for 20% to 40% of cases of severe hepatic failure. Hence, innovative methods must be employed to create a fresh formulation of ORL that functions as a dietary supplement for weight loss. [6].

Additionally, there has been a link between liver damage and the natural weight-loss product Hydroxycut. There have been reports of liver damage even though the components in Hydroxycut have changed over the past ten years [4]. The use of dietary supplements has doubled in the US, with 18.9% of persons reporting to using them in 2019 [7]. The growing prevalence of dietary supplements can be attributed to consumers' heightened awareness of overall health and their inclination to prevent diseases through improved nutritional status, coupled with the belief that these treatments are safe. [8].

Health warnings are made about these items, but the general population might not follow them since they don't know the risks associated with ingesting an unregulated chemical that is advertised as having health benefits. Many products bearing the name Hydroxycut are still readily accessible and in widespread use today [9].

Aims of the study

The purpose of this study is to evaluate and clarify the effects of Hydroxycut and Revit Orlistat on male albino rats' livers, as well as the consequences of using these medications on the livers of albino rats. The effects it has on liver health and the risk of liver injury. Additionally, measuring the levels of some physiological markers, such ALT and AST, and contrasting the outcomes with the control group.

Materials and methods

The study samples included 30 blood samples taken from male of albino rats and divided the rats into 6 groups, each consisting of 5 rats and one of groups was a control group that they were given (normal saline). The albino rats used were two months old and their body weight was 180-225 grams. The treatment period continued for a month until the weight of each rat reached (130-150) grams. Albino rats in good health were obtained from the animal

house at the College of Veterinary Medicine, Tikrit University. The estimation of serum levels of AST and ALT was by using a commercial kit in the form of a ready-made analysis kit.

The liver biopsies of the rats were placed in a fixative solution containing 10% formalin. Subsequently, the sample was subjected to regular processing and stained using Hematoxylin and Eosin (H and E). Proforma was used to collect and document clinical data. Microscopic examination was conducted on all cases tissue.

Sampling

The animals' weight was measured both before and after administering the dose using a highly sensitive scale. Blood samples (ranging from 2.0 to 5.0 ml) were collected immediately after sacrificing the animals by puncturing their hearts. The samples were then placed in gelatinous tubes and allowed to clot. The serum was isolated using centrifugation at a speed of 3000 revolutions per minute for 15 minutes. The serum is stored at a temperature below 20 degrees Celsius, which is the freezing point. Intended for utilization in physiological and biochemical examinations [10].

A colony of albino rats was bred and nourished on a specific sort of specialized animal diet (Balanced diet) throughout the experiment.

The animals are dissected, and after the experimental drugs are given to the white rats, liver tissue is taken out. This organ is then preserved with a formalin solution (10%). Following that, it receives routine care and is stained with hematoxylin H and eosin (E). The samples were examined under a microscope to evaluate the different histological patterns and determine how these drugs affected the tissue of the organ.

Inclusion criteria: These were based on the following features:

- Rats are overweight.
- Albino rats in good health.

Exclusion criteria: These were based on the following:

- Rats weighing less than 120 grams.
- Rats with medical problems such as liver or kidney failure.

Evaluation of AST and ALT serum concentrations

The estimation of serum levels of AST and ALT was done by using the commercial kit in the form of a ready-made analysis kit.

Statistical analysis

The statistical analysis performs using SPSS version 22. The AST and ALT values were subjected to statistical analysis using the Minitab computer, specifically the Analysis of Variance (ANOVA) tool in SPSS version 22. The arithmetic means were compared using the Duncan multiple range test to assess the disparity between the two groups. This involved testing the mean and standard deviation (SD) at a significance level of 0.05. Additionally, the variation in the weights of the rats was calculated with program (SPSS version 22) [11,12].

The experiment includes the use of 30 male albino rats divided into 6 groups, each group consisting of 5 rats:

The first group:

It includes 5 rats who were considered a control group and were given 1 ml of physiological saline solution as a solvent (distilled water) and taken for 20 days. as shown in Figure 1

The second group:

It includes 5 rats who were given the organic solvent Dimethyl sulfoxide (DMSO) and took it for 10 days. as shown in Figure 2

The third group (the first treatment group):

It includes 5 rats and they were given the first treatment group, which is the drug Revit (Orlistat) at a concentration of 0.3 milligrams dissolved in 5 ml of distilled water. 1 ml of the solution is given to each rat and taken for 20 days. as shown in Figure 3

The fourth group:

It includes 5 rats and they were given the second treatment group, which is the drug hydroxyketo at a concentration of 0.68 milligrams and dissolved in 5 ml of the solvent DMSO. Each rat is given 1 ml of the solution and takes the treatment for 10 days. as shown in Figure 4

The fifth group:

It includes 5 rats given the first supra-therapeutic group, which is the drug Revit (Orlistat) at a concentration of 0.4 milligrams dissolved in 5 ml of distilled water. Each rat is given 1 ml of the solution and takes the treatment for 20 days. as shown in Figure 5

The sixth group:

It includes 5 rats who were given the lethal dose, which is the drug hydroxyketo at a high concentration of 1.42 milligrams dissolved in 5 ml of the organic solvent (DMSO). Each rat is given 1 ml of the solution for 10 days. as shown in Figure 6.

Results

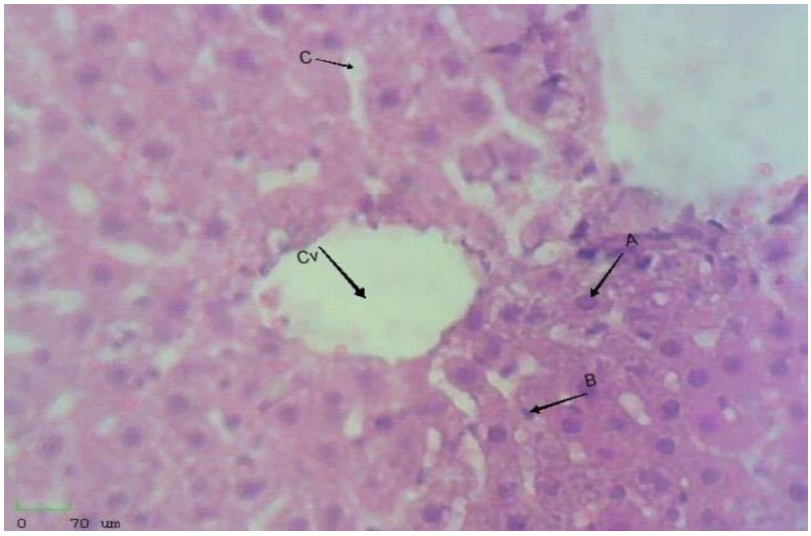

Fig. 1 A cross-section of the liver of a control rat, showing a central vein (Cv-central vein) and hepatocytes. A-hepatocyte, B-kupffer cell) and hepatic sinusoids (C-sinusoid.), (H and E) 400 x.

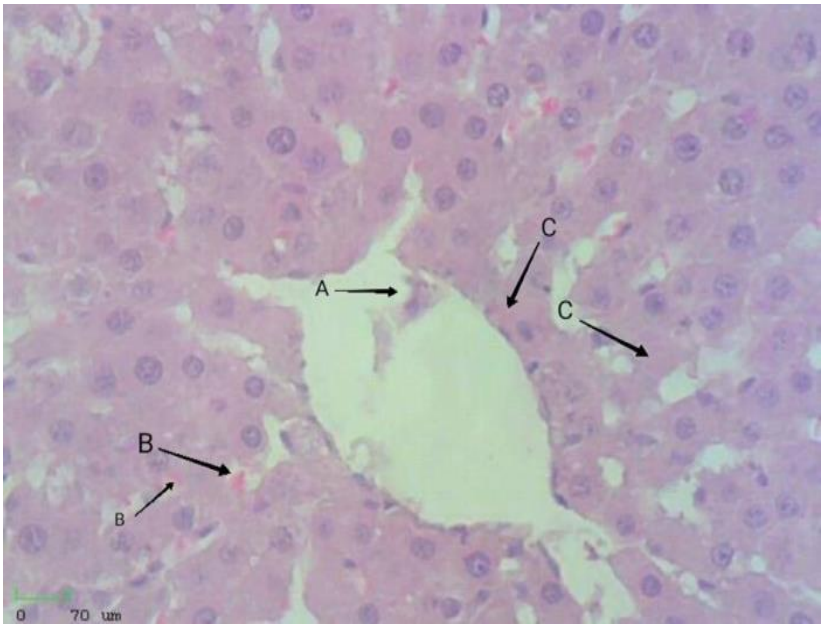

Fig. 2 A cross-section of the liver of a control rat (DMSO) for 10 days, showing peeling of the central vein wall .A-desquamation of the wall of central vein), (B-hemorrhage), and necrosis (C-Necrosis). (H and E) 400 x.

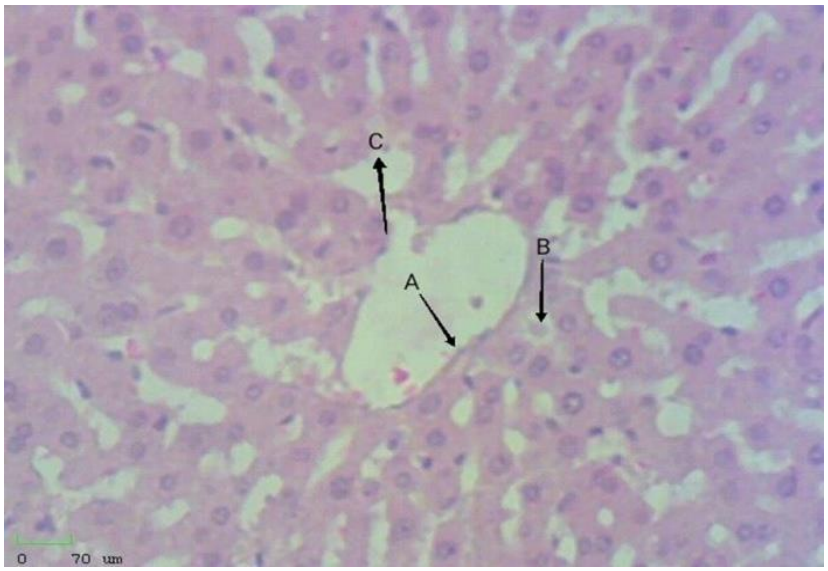

Fig. 3 A cross section of the liver of a rat treated with the drug Revit (Orlistat) at a concentration of 0.3 mg for 20 days. A-desquamation in central vein, B-necrosis, and C-Kupffer cells, (H and E) 400 x.

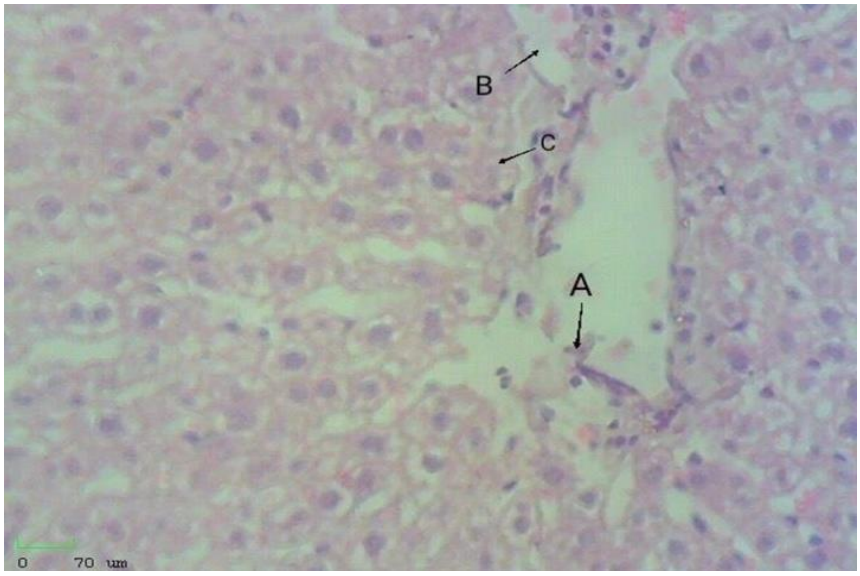

Fig. 4 A cross-section of the liver of a rat treated with hydroxyketo at a concentration of 0.68 mg for 10 days, showing (A-desquamation and damage in the central vein, (B-hemorrhage), and necrosis (C-Necrosis), (H and E) 400x.

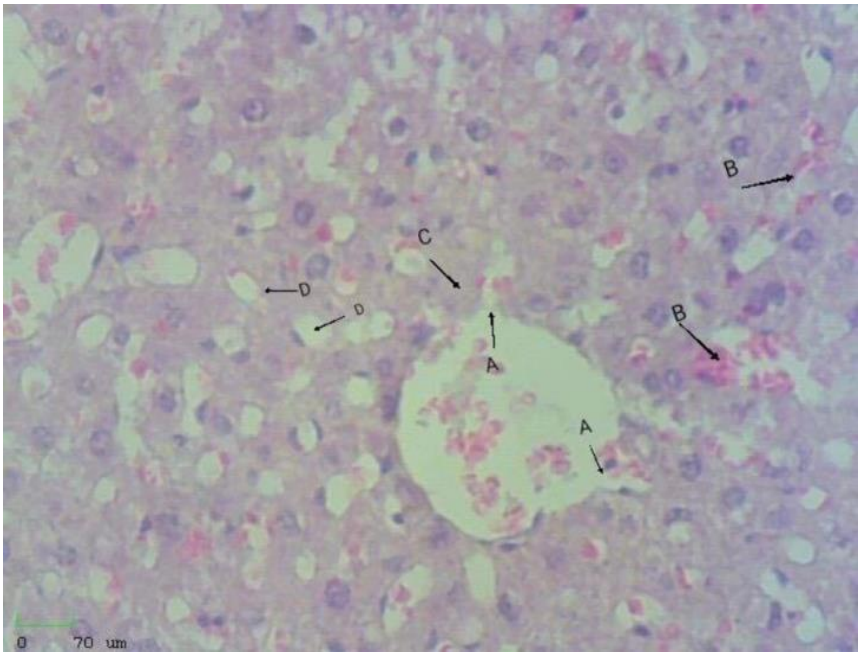

Fig. 5 A cross-section of the liver of a rat treated with the drug Revit (orlistat) at a concentration of 0.4 mg for 20 days, showing (A-desquamation in the central vein, bleeding) (B-hemorrhage), and necrosis(C-Necrosis) (D-vacuolization of hepatocyte), (H and E) 400 x.

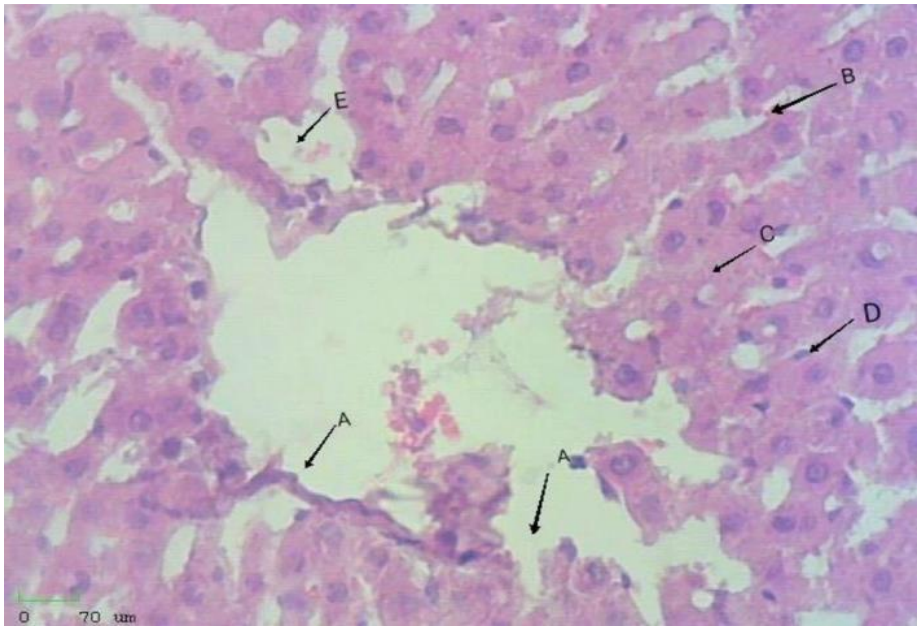

Fig. 6 A cross-section of the liver of a rat treated with hydroxyketone at a high concentration of 1.42 mg for 10 days, showed desquamation of the wall of the central vein, (A-hemorrhage), and necrosis (C-Necrosis and dilatation of sinusoids (E-dilation in sinusoid) and Kupffer cells (D-Kupffer cells), (H and E) 400 x.

1-Glutamic-oxaloacetic transaminase (GOT) level concentration in the blood serum of albino rats (alanine aminotransferase (ALT)

The findings of the present investigation, as depicted in Table (1) and Figure (7), revealed a notable elevation in the concentration of (GOT) in the serum of albino rats as compared to the control groups. Furthermore, there were statistically significant variances at a probability threshold of ($P < 0.05$).

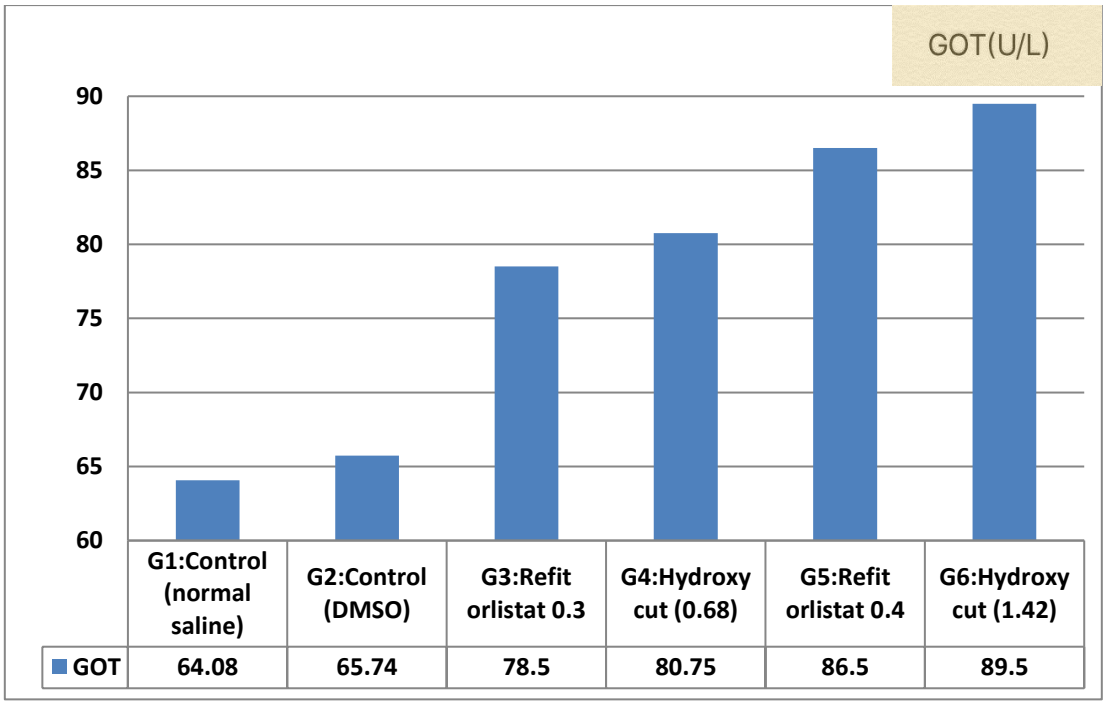

Fig. 7 Assessment of serum level ALT of white rats (U/L) in studied groups

2-Glutamate-pyruvic transaminase (GPT) level concentration in the blood serum of albino rats (aspartate aminotransferase (AST)

The findings of the present investigation, as depicted in Table (1) and Figure (8), indicate a notable elevation in the concentration of (GPT) in the serum of albino rats as compared to the control groups. Moreover, there were statistically significant variances observed at a probability threshold of ($P < 0.05$).

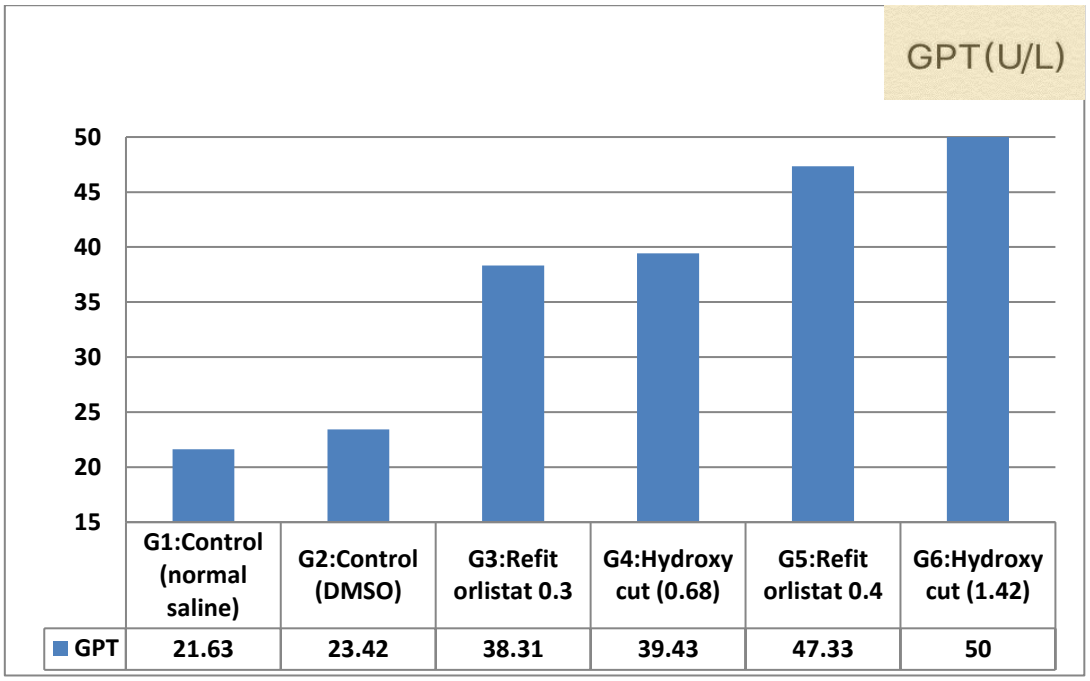

Fig. 8 Assessment of serum level AST of white rats (U/L) in studied groups

Table 1. Mean and Standard Deviation± of ALT and AST:

NO.	Group	N	AST/GOT(U/L)		ALT/GPT(U/L)	
1	G1	5	64.08 ±1.30	c	21.63 ±0.46	c
2	G2	5	65.74±1.55	c	23.42± 0.68	c
3	G3	5	78.50±2.988	b	38.31±1.13	b
4	G4	5	80.76 ±3.862	b	39.43 ±1.15	b
4	G5	5	86.50±4.550	b	47.33±1.35	b
6	G6	5	89.50 ±4.981	a	50.00±1.92	a
P value			P<0.05		P<0.05	
G1: Control (normal saline)			G3:Refit orlistat 0.3		G5:Refit orlistat 0.4	
G2: Control (DMSO)			G4:Hydroxycut (0.68)		G6:Hydroxycut (1.42)	

Discussion

The results of this study supported those of Ahmed et al. (2021) [13], who found that obese rats given orlistat showed significantly higher GOT and GPT activity levels than the healthy control group. Since the rise of these markers is a result of tissue damage caused by toxic substances or pathological illnesses, their levels are important in both clinical and toxicological contexts.

The results of these were also consistent with another study in which the effect of hydroxycut treatment used for weight loss on rats was determined, and these rats showed acute liver failure [14].

Aminotransferases are enzymes that facilitate the transfer of an amino group from amino acids to oxoacids through a biochemical reaction called transamination. Assaying these enzymes is commonly conducted to identify liver disorders [15]. Liver enzymes are commonly used as a sign of liver dysfunction. A liver biopsy is also used as the gold standard for diagnosis. Any damage to liver cells increases the activity of these enzymes in the liver before they are transported into the bloodstream, thus increasing the levels of enzymes in the serum. This coincides with the results of... Another study reached this same result [16] , in which an increase in the activities of ALT and AST enzymes was detected in albino rats, indicating liver damage. However, there was an undesirable effect on liver function tests, suggesting possible liver toxicity after long-term administration [17].

Alternatively, orlistat medication impacts pancreatic lipase, the enzyme responsible for metabolizing lipids in the intestine. Orlistat therapy inhibits the action of lipase, preventing the hydrolysis of triglycerides in the diet into absorbable free fatty acids. As a result, these triglycerides are expelled without being digested. Orlistat is a saturated derivative of lipstatin, which is a powerful natural inhibitor of pancreatic lipase that is obtained from the bacterium Streptomyces toxytricini. Nevertheless, due to its uncomplicated nature and consistent performance, orlistat was chosen over lipstatin for the development of an anti-obesity medication. Due to its ability to decrease the assimilation of dietary fat by approximately 30%, this medication induces weight reduction that is comparable to or surpasses the results achieved by adherence to a diet low in fat. [15].

Orlistat is generally well accepted and received approval from the Food and Drug Administration in 1998. Its safety is substantiated by a comprehensive collection of data [17]. Nevertheless, controlled clinical trials have documented instances of significant liver damage and hepatic failure. A clinical trial was conducted where individuals who were administered Orlistat had to stop the treatment because they experienced adverse effects caused by the medicine [18].

Conclusion

The study showed that Hydroxycut and Refit orlistat have a direct negative effect on liver tissue, especially if taken at a dose above the therapeutic level in albino rats and causing acute liver failure. Therefore, it is important to consider their toxicity profile while prescribing, especially in elderly patients and kidney patients. And the liver. There is a positive relationship between the use of the two drugs and the body mass index in albino rats, and thus a decrease in the weight of the treated rats.

Conflict of interest: No conflict of interest**.**
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Author contribution: Authors contributed equally in the study.

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التقييم النسيجي والفسيولوجي للتسمم الكبدي بالهيدروكسيكت والريفيت أورليستات في ذكور الجرذان البيضاء

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معلومات المؤلف
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الخلاصة:
أصبحت السمنة شائعة في جميع أنحاء العالم، حيث تهدد حياة الناس اليومية وتهدد أيضًا صحة الحيوان بالإضافة إلى زيادة خطر الإصابة بالأمراض المرتبطة بالسمنة. اكتسبت المكملات الغذائية مثل Hydroxycut وRefit orlistat التي تستهدف السمنة شعبية وأصبحت الآن سببًا شائعًا لضعف الكبد الناتج عن الأدوية والتسمم الكبدي. تهدف هذه الدراسة إلى تقييم تأثير الهيدروكسيكت والريفيت أورليستات على الكبد نسجياً وفسيولوجياً. تم استخدام ذكور الفئران البيضاء في التحقيق الحالي الذي استمر من 29 أغسطس 2022 حتى 29 أبريل 2023. وتم استخدام ثلاثين عينة دم من ذكور الفئران البيضاء في الدراسة. تم إنشاء ست مجموعات مكونة من خمسة فئران لكل منها من تصنيف الفئران. وكانت إحدى المجموعات بمثابة المجموعة الضابطة. أظهرت نتائج الفحص النسيجي لكبد الجرذان تغيرات مختلفة، تراوحت بين ظهور تقشر جدار الوريد المركزي، والنزيف، وتفريغ خلايا الكبد، وخلايا كوبفر، والنخر أيضاً. حددت هذه الدراسة تركيزات AST وALT في مصل الدم وأظهرت ارتفاعًا ملحوظًا في كليهما. وعلاوة على ذلك، كانت هناك فروق ذات دلالة إحصائية عند مستوى احتمال أقل من 0.05. وأظهرت الدراسة أن هيدروكسيكت وأورليستات لهما تأثير ضار مباشر على أنسجة الكبد، مما يؤدي إلى تطور فشل الكبد الحاد. لذلك، من المهم مراعاة مدى سمية هذه الأدوية أثناء وصفها، خاصة عند المرضى المسنين ومرضى الكلى والكبد.