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Effect of High-Fat Diet on the Kidneys of Albino Rats and the Protective Role of Beetroot Juice Compared with Orlistat

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Abstract

The current study aimed to clarify the physiological and histological damage resulting from the high-fat diet in the kidneys of white rats and the protective role of beet root juice, Beta Vulgaris, in comparison with the drug Orlistat. The study used 35 rats distributed in plastic cages in seven groups, 5 rats for each group. The first group included the healthy control group, the second group the group dosed with high-fat food, the third group dosed with beetroot juice (4ml/kg), the fourth group dosed with orlistat (10ml/kg) by mouth, and the fifth group dosed with high-fat food and beetroot juice (4 ml / kg), and the sixth group dosed with high-fat food and Orlistat (10 ml / kg), and the seventh group dosed with high-fat food and beet root juice (4 ml / kg) and Orlistat (10 ml / kg),

The results showed a significant increase in the concentration of urea, creatinine, estrogens, ghrelin, and MDA for the group of rats fed with a high-fat diet. And a significant decrease in the concentration of SOD, CAT, as shown by the histological sections of the kidney tissue of the group treated with a high-fat diet, showing the destruction of the renal glomerulus (DG) and the disintegration of the visceral tissue around the blood vessel (DP) with the presence of hemorrhage within the kidney tissue (H) and thickening of the vessel wall (TW) and the group treated with beet juice at a concentration of (4 ml/kg) showed a significant decrease in the concentration of urea, estrogens, and antioxidants (MDA, SOD, and CAT.(

The histological sections of the kidney tissue of the group treated with beetroot juice showed the renal glomerulus (G), the proximal convoluted tubule (PCT) and the distal convoluted tubule (DCT), and the space around the glomerulus. The group fed with orlistat 10ml showed a significant increase in the concentration of salts, estrogens, and MDA, and a decrease in the concentration of SOD, ghrelin, and leptin. and the histological sections of a section of kidney tissue of the group treated with orlistat showed swelling of the renal glomerulus (SG), bleeding within the kidney tissue (H), and narrowing of the space around the glomerulus. and The group fed a high-fat diet and beetroot juice showed a significant increase in the concentration of electrolytes and estrogens, and a significant decrease in the concentration of urea, ghrelin, leptin, and antioxidants (MDA, SOD). The histological sections of the kidney tissue in the group treated with a high-fat diet and beetroot juice showed Shown is the renal glomerulus (G), the proximal convoluted tubule (PCT), and the distal convoluted tubule (DCT), and the space around the glomerulus (C). The group fed a high-fat diet and orlistat showed a significant increase in the concentration of electrolytes and estrogens, and a significant decrease in the concentration of urea, leptin, ghrelin, and antioxidants (SOD). The histological sections of the kidney tissue were Shows the renal glomerulus (G), swelling of the cells lining the proximal convoluted tubule (SW), and degeneration of the cells lining the distal tubule (D), with hemorrhage within the renal tissue (H), and the group fed a high-fat diet, beetroot juice, and orlistat showed a significant decrease in the levels of urea, creatinine and leptin, and a substantial increase in the levels of E2 estrogens. The histological sections of the kidney tissue in

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the group treated with a high-fat diet and orlistat showed that the lining of the renal tubules (UT) shows detachment with hemorrhage within the renal tissue (H).

Introduction

Approximately 90% of obese people suffer from kidney disorders, such as an increase in urea, creatinine, and antioxidants, including MDA, an increase in the hormone ghrelin, and a decrease in leptin levels.Since it is known that fats are organic compounds containing hydrogen, carbon and oxygen atoms, and they form the framework for the structure and function of living cells.(Khanday, 2019) It also contains fatty acids and their derivatives such as steroids, terpenes, carotenoids, and bile acid, which are non-polar and have the ability to dissolve in organic solvents such as diethyl ether, hexane, benzene, chloroform, or methanol (Akoh., 2017). An increase in fat leads to Hyperlipidemia, which is an abnormal increase in the level of lipoproteins (Hyperlipoproteinemia)that transport lipids in the blood such as very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), and low levels of high-density lipoproteins (HDL) (Machaba, 2014; Mishra et al., 2011)Abnormal cholesterol levels are the result of an unhealthy lifestyle including a high-fat diet and other lifestyle factors such as being overweight and smoking (Gupta et al., 2016; Hussein et al., 2014).Also, there are other factors such as diabetes mellitus, kidney disease, pregnancy, polycystic ovary syndrome, and some medications such as (diuretics, beta-blockers) and medications that treat depression as well, which have been shown to increase cholesterol levels (Ankur et al., 2012; Kelly, 2010.)

In cases of oxidative stress, it causes an increase in free radicals, which causes an increase in oxidation, and thus the lipoprotein lipase enzyme present in the various tissues of the body decreases.This decrease causes an imbalance in lipid levels and an increase in the concentration of triglycerides in the blood (Stohsj etal., 2000.)Beetroot is a plant known as Beta vulgaris L. (family Chenopodiaceae). This plant is commonly cultivated in all countries of the world for its edible root and is used for its many powerful antioxidant properties (Winkler et al. 2005).It is also a source of nitrates, vitamins, minerals, and the water-soluble nitrogenous pigments, betalains, which contain two major classes, red betacyanins and yellow betaxanthin (Lee et al. 2006) Beetroot has a long history of medicinal uses and is used in traditional medicine to treat cancer and jaundice. These roots also possess carminative, emmenagogue, hemostatic, and nephroprotective properties. Several investigations have shown that beetroot improves several diseases such as type 2 diabetes, atherosclerosis, high blood pressure, and dementia (Winkler et al.2005; Curtis et al. 2015) .Since beetroot is rich in dietary nitrates, it is widely used to treat cardiovascular diseases (Lundberg et al., 2008)Red beetroot has been ranked among the ten most powerful antioxidant vegetables. The antioxidant capacity of red beetroot is closely related to the betalain content .Previous studies have shown several health benefits of beetroot on age-related diseases including high blood pressure, diabetes and high blood cholesterol.The beneficial effects of beetroot are attributed to the presence of high amounts of NO3 and its effects on the nitrate-nitrite-nitric oxide (NO) pathway (Sayyar et al., 2022). Orlistat, an anti-obesity drug, has been used as a therapeutic modality and is practically prescribed to obese individuals with a BMI greater than 30 kg/m2 and those who cannot achieve their goals in lifestyle and nutritional interventions. Orlistat has very mild side effects on the digestive system, particularly it may cause diarrhea, indigestion and flatulence. It has been recognized as one of the anti-obesity drugs approved by the Food and Drug Administration (FDA) for long-term use. Orlistat acts selectively towards gastrointestinal lipase by preventing the

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hydrolysis of ingested dietary fat into absorbable free fatty acids and glycerol. For this reason, orlistat has been shown to be effective in improving obesity parameters such as body mass index, lipid profiles, white adipocyte volume, and fecal fat excretion in animal models, as well as complications of obesity such as metabolic syndrome and endothelial dysfunction in humans.

Materials and methods

The beet plant, Beta Vulgaris, was collected in December and January of 2021 in Baghdad Governorate, central Iraq. Work began by washing the samples well with water from dirt, dust, and impurities adhering to them. The samples were peeled and cut into small pieces to conduct the squeezing process and obtain juice pure of impurities. After filtering it well.

juicing process

Grains were taken from the fresh Iraqi beet plant, Beta Vulgaris, and washed well in water to remove dust and impurities, and then the outer part of it was removed (peeled) by a manual peeler.After that, it was washed in sterile water, then cut into small pieces with a knife, and the small pieces were placed in an electric mixer, and the pieces were mixed until they became thick and well-coherent. This thick-bodied solution was filtered by multiple pieces of medical gauze to prevent the impurities that were not well mashed from coming out of the mixture. In this way, the juice of the beetroot plant was obtained. It was re-filtered by filter papers to obtain a very pure juice without any addition after it This juice was placed inside a well-tight glass container, and then placed in an electric refrigerator until the juice was used (Amnah, 2013).

Animals used in the study

In this study, (35) albino female rats, Sprague Dawley, were used in this study, their ages ranged between 4-5 months, obtained from the College of Veterinary Medicine at the University of Tikrit, and their weights were 170-200 gm, as they were raised in the animal house unit of to the College of Veterinary Medicine / University of Tikrit, and after being examined by the specialist doctor in the animal house, they were placed in plastic cages, dimensions of (46 x 28 x 13 cm). Sawdust was placed at the bottom of the cages. These animals were subjected to the appropriate laboratory conditions of temperature (and an almost equal light period), taking into account the ventilation factor.The hygiene aspects of the cages were taken into account, sterilized, and sawdust replaced twice a week.The cages were sterilized with disinfectants (90% Dettol, 70% potassium permanganate and alcohol) and the animals were left for two weeks to adapt to the new conditions and to ensure that they were free of diseases. Food and water were given continuously and in sufficient quantities (Al-Janabi, 2008).It was fed with the diet allocated for it and in the following proportions, which are yellow corn 35%, wheat 35%, soybeans 20%, concentrated protein 10%, dry milk 1%, in addition to preservatives and antifungals (Al-Janabi, 2008), and they were given water freely during the experimental period (60 days).The experiment began on 12/9/2021 and ended on 2/9/2022. As for the animal, which was fed a high-fat diet, the diet consisted of all vegetable oil mixed with the diet. Each (8 kilograms) had a diet (2 liters), and the total diet was 18 kilograms and the total vegetable oil. (5 liters). All the animals were weighed at the end of each week of the experiment for the purpose of measuring their weight and identifying the change in it due to the effect of diet, juice and drug.

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Distribution of Study Animals

The animals that were used in the experiment were divided into seven groups, with (5) animals for each group, as follows:

1-The first group (Control -ve): the healthy group that was given

Ordinary drinking water and food for 60 days.

2- The second group (Control+ve): the infected group that was given the high-fat diet for 60 days.

3- The third group: the group treated with beetroot juice, Beta vulgaris, this group was given for 60 days (4 ml of body weight.(

4 -The fourth group: drug treatment This group was given Orlistat by mouth at a concentration of ((10ml of body weight.(

5 - Fifth group: This group was dosed with a high-fat diet and beetroot juice at a rate of (4 mg/kg) of body weight during the experimental period.

6- The sixth group: the animals of this group were dosed with a high-fat diet and the drug Orlistat at a rate of 10 mg/kg of body weight by mouth for 60 days.

7- The seventh group: This group was given a high-fat diet and beetroot juice at a concentration of (4 mg/kg) of body weight and Orlistat at a rate of 10 (mg/kg) of body weight for 60 days.

Collection of Blood Samples

After the end of the experiment, which lasted for 60 days, and after 24 hours had passed since the last dose, the animals were killed, blood samples were collected from the animals through a heart attack, and the samples were placed in plastic tubes free of anticoagulant, then the samples were transferred to a centrifuge to separate the serum at a speed of 3000 cycles/minutes for 15 minutes, and then using micropipettes, and the obtained serum was distributed into four parts in Eppendorf tubes (to avoid repeated freezing and thawing of the sample) and kept at a temperature (-20 °C) to conduct hormonal tests for obesity (Ghrelin, Leptin) and hormones female (estrogen E2), blood parameters CBC, antioxidants (MDA, GSH, SOD, CAT), biochemical tests that included lipid profiles (TC, TG, HDL, LDL, VLDL), liver function enzymes (TSB, GOT, GPT, ALP) and kidney function (CRE, UR) **.**

Leptin Hormone

The assay kit from Fine test/china for the leptin hormone was used by the ELISA device (Enzyme-linked immune sorbent assay).

principle

This kit was based on the screening technique of sandwich enzyme-linked immunosorbents. The immobilized anti-Ab antibody was pre-coated on a 96-chamber plate. A biotin-conjugated antibody was used as a detection antibody.Standards, test samples, and biotin-conjugated detection antibodies were added to the wells subsequently and washed with wash buffer, HRP-Streptavidin

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was added and unbound conjugates were washed with wash buffer. TMB substrates were used to visualize the enzymatic reaction of HRP, TMB was catalyzed by HRP to produce a blue color product that changed to yellow after the addition of an acidic stop solution. The intensity of the yellow color is proportionalWith the target amount of sample captured in the plate, read O.D. The absorbance is at 450 nm in a microplate reader, and then the target concentration can be calculated.

Procedure

When all samples and reagents are diluted, they must be mixed thoroughly and evenly. Before adding TMB into the chamber, calibrate the TMB substrate for 30 min at 37 °C, drawing a standard curve for each test is recommended.

1-Set the standard, test samples (diluted at least 1/2 with sample dilution solution), and control sample (blank) chamber on the pre-coated plate respectively, it is recommended to measure each standard and sample in duplicate, we wash the plate twice before adding the chambers standard, test sample, and blank wells (blank).

2-Standards setup: Aliquot 100ul of Zero tubes, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube, and Sample Dilution Buffer (blank) in standard chambers.

3-100ul of the properly diluted sample has been added to the test sample chamber.

4- Incubate and cover the plate with a seal and incubate it at 37°C for 90 minutes.

5-We remove the cover and empty the contents of the plate. We wash the plate twice using Wash Buffer, taking into account that the chambers are not completely dry at any time.

6- Biotin-labeled antibody: We add 100 ul Biotin to a Biotin-labeled antibody solution in the aforementioned quarantine (standard, test sample, and blank wells). We add the solution to the bottom of each chamber without touching the side wall, cover the plate and incubate at 37 °C for 60 min.

7-Washing: We removed the cover, and washed the plate 3 times with Wash Buffer, leaving the Wash Buffer in the chamber for 1-2 minutes each time.

8-We added 100ul of this working solution in each chamber, covered the plate, and incubated it at 37°C for 30 minutes HRP-Streptavidin Conjugate

9- Washing: We removed the cover and washed the plate 5 times with the Wash Buffer, leaving the Wash Buffer to wash in the chamber for 1-2 minutes each time.

10- We added 90ul of TMB Substrate in each chamber, covered the plate and incubated at 37 °C in the dark during 10-20 minutes, (Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. We can finish the reaction When an apparent gradient appears in the standard rooms) TMB Substrate

11-Stop: We added 50ul stop solution in each chamber. The color immediately turned yellow. The order of addition of the stop solution should be the same as that of the TMB substrate.

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12- O.D. Measurement:We read the OD of absorbance at 450 nm in the Microplate Reader immediately after addition of the stop solution, with respect to the calculation, (relative OD.450) = (450 OD of each chamber) - (450 OD of the empty chamber), the standard curve can be plotted as O.D.450 The relative concentration of each standard solution (Y) versus the corresponding concentration of the standard solution (X). The target concentration of the samples can be gleaned from the standard curve.

Ghrelin Hormone

The assay kit from Fine test/china company for the hormone ghrelin was used by the ELISA device (Enzyme-linked immune sorbent assay(

principle

This group was based on the Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with the objective. During the reaction, the target competes in standard competitions with a fixed amount of the target on the solid surfaces on the target's Biotinylated detection antibody.Excess unbound and standard conjugate samples are washed from the plate, and HRP-Streptavidin (HRP) is added to each well and incubated microplate. Then a TMB substrate solution is added to each chamber, the enzymatic substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured by a spectrophotometric method at a wavelength of 450 nm. The target concentration in the samples is then determined by comparing the OD of the samples to the standard curve.

Procedure

When diluting samples and reagents, they must be mixed thoroughly and evenly. Before adding TMB into the chambers, titrate Substrate TMB for 30 minutes at 37 °C.

1- We set the standard compartments, test and control samples (blank) on the pre-coated plate in succession, and then recorded their locations. It is recommended to measure both the standard and sample in duplicate. We washed the plate twice before adding standard, sample, and control (blank) wells.

2- We added the sample and biotin-labeled antibody: 50 ulstandard, blank or sample was added to each chamber, the empty well was added with sample/standard dilution solution. We immediately added 50ul Biotin labeled antibody working solution into each compartment. We covered it with the clapboard wrap we provided. Gently pressing the plate to ensure thorough mixing, we placed it in the incubator for 45 minutes at 37 °C.

3- Washing: We removed the cover, washed the plate 3 times with Wash Buffer, leaving the wash buffer in the wells for 1 min each time. After the last wash, we removed any remaining wash buffer by aspirating or decanting.

4-HRP-Streptavidin Conjugate: We added 100ul of this working solution in each chamber that we covered with a new cover. Incubate for 30 minutes at 37 °C.

5-Washing: We removed the cover and washed the plate 5 times with Wash Buffer, leaving the wash buffer in the wells for 1-2 minutes each time.

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6-Substrate TMB: We added Substrate TMB 90ul in each compartment, covered the plate and incubated at 37 °C in the dark for 10-20 minutes.

7- Stop: We added Stop Solution 50ul in each chamber, the color will turn yellow immediately The order of adding Stop Solution should be the same as that of Substrate TMB.

8-OD measurement: We read OD. Absorbance at 450 nm in a Microplate Reader immediately after addition of the stop solution. In terms of calculation, the standard curve can be plotted as O.D.450 for each standard solution (Y) versus the corresponding concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve.

Preparation of histological sections

After the removal of the organs, which are the kidneys, they were fixed directly in 10% formalin solution for 24 hours. Then, the histological sections of the rats' organs were prepared, according to what was reported by Bancroft and Al-Tarda et al 2003), which were determined by the following processes:

The samples were washed with tap water for one hour to remove the fixative from the excised tissues, then these washed samples were passed to different concentrations of ethyl alcohol in ascending order 70%, 80%, 90%, 95%, and 100% for half an hour for each concentration except absolute alcohol for two times each time Half an hour to withdraw water particles or dehydration, and to make the samples more transparent and clear, they move to the clearing stage by placing the samples with xylene for a period of (30-45) minutes, followed by the infiltration stage, as the tissue pieces were passed with a mixture of xylene and Paraffin Wax has a melting point of (58-60 °C) at a ratio of 1:1 and the mixture is placed in an electric oven at a temperature of 60 °C for (15) minutes.

Followed by the embedding stage, where the molten paraffin wax was placed in iron molds special for this stage in the shape of the letter (L), and the information about the organ was written on a sheet of paper and placed on the mold, and the bubbles were removed with a hot needle, then left in the refrigerator to harden and cool completely.These molds were placed in a rotary microtome with a thickness of (5) micrometers, and the strips were placed with a dissection needle and spread over water in a water bath at a temperature of 37-40 degrees Celsius .Then the sections were loaded onto the glass slide marked with a diamond pen, after that the slides were passed on xylol for 5-10 minutes to remove the paraffin wax, then left to dry .After that these slides were passed on several concentrations of ethyl alcohol, but in a descending manner, 100%, 95%, 70%, and 35% to remove the xylol, for a period of (5) minutes in each concentration, then a drop or two of hematoxylin stain was added for 7-8 minutes, followed by eosin stain for time30-60 seconds

Then the glass slides are re-passed in ethyl alcohol at increasing concentrations (70-100%) for two minutes, and the purpose is to completely remove the water molecules. The final stage is to percolate the tissue slides with xylene for 10 minutes, then leave them to dry. (D.P.X) was used for docking as it dries faster, then it was covered with a cover slip, after which the slides were dried on a hot plate at 40 degrees Celsius.

The samples were examined by Olympus light microscope, and the histological sections were taken with a Samsung digital camera.

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Statistical analysis :

The results were analyzed statistically by using Analysis of Variance (ANOVA), and the arithmetic means of the coefficients were compared using Duncan's multiple range test at a significant level ($p \leq 0.01$) (Al-Rawi, 1984).

Results

Results of kidney function levels

Table (1) shows a significant increase in the levels of urea and creatinine in the group fed with high-fat food and a significant decrease in the group fed with high-fat food and beetroot juice, and the group fed with orlistat drug. Table (1) shows a significant increase in the levels of salts in the group fed with high-fat food and beet juice A high-fat food group and orlistat

the group	UREA	CREATININ	U. ACID
the control	29.88 ± 3.43 c	0.4040 ± 0.047 b	1.244 ± 0.1197 C
High fat food	42.26 ± 3.87 A	0.6920 ± 0.0864 a	1.200 ± 0.251 C
Beet root juice	34.58 ± 1.25 B	0.4200 ± 0.0604 b	1.596 ± 0.521 Bc
Orlistat	31.92 ± 2.71 C	0.3820 ± 0.0466 bc	1.682 ± 0.415 Ab
High fat food and beetroot juice	22.88 ± 2.43 E	0.4450 ± 0.0975 b	1.918 ± 0.662 A
High fat food and drug	26.08 ± 4.82 D	0.4380 ± 0.0779 b	1.970 ± 0.542 A
orlistat	26.77 ± 1.72 D	0.3400 ± 0.0656 c	1.247 ± 0.573 C
High-fat diet, beetroot juice, and orlistat	1.247 ± 0.573 C	0.3400 ± 0.0656 c	26.77 ± 1.72 D
P-Value	0.0007 **	0.0005**	0.047 *
-Value-F	18.43	12.42	2.29

Sexual Hormones

Results of estrogens: Table (1) showed a significant increase in the level of estrogens in the group fed high-fat diet, the group fed high-fat diet and orlistat, and the group fed high-fat diet and beetroot juice.While it showed a significant decrease in the group fed beetroot juice.

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leptin hormone

Table (2) results show a significant increase in leptin levels in the group fed high-fat diet and beetroot juice, and the group fed high-fat diet and orlistat, and a significant decrease in the group fed beetroot juice.

ghrelin hormone

The results of ghrelin levels are shown in Table (2), showing a significant increase in the group fed with high-fat food and a significant decrease in the group fed with orlistat.

Antioxidant

Table (4-4) shows a significant increase in the concentration of malondialdehyde in blood serum (MDA) in the group fed with a high-fat diet, and a significant decrease in the group fed with beet root juice, and the group fed with orlistat.Table (4-4) shows a significant decrease in the concentration of glutathione in the group fed with a high-fat diet, and a significant increase in the groups fed beetroot juice, and the drug Orlistat.The results of the enzyme Super Oxide Dismutase SOD in Table (4-4) show a significant decrease in the experimental groups, except for the group fed with high-fat food, beet root juice and orlistat, did not show a significant difference, while the CAT enzyme in Table (4-4) shows a significant decrease in the group fed with high-fat food and an increase Significantly increased in groups fed beetroot juice and orlistat.

the group	CAT	SOD	GSH	MDA
the control	51.855 ± 0.871 B	9.494 ± 0.260 a	0.389 ± 0.012 a	2.88 ± 0.488 c
High fat food	42.360 ± 0.410 C	7.898 ± 0.253 c	0.256 ± 0.025 c	6.156 ± 0.791 a
Beet root juice	52.373 ± 0.489 Ab	8.551 ± 0.196 b	0.343 ± 0.021 b	1.885 ± 0.381 d
Orlistat	53.170 ± 3.290 A	8.792 ± 0.209 b	0.393 ± 0.038 a	4.549 ± 1.504 b
High fat food and beetroot juice	52.130 ± 0.267 Ab	8.529 ± 0.229 b	0.342 ± 0.027 b	2.156 ± 0.115 d
High fat food and drug	53.332 ± 2.020 A	8.837 ± 0.866 b	0.354 ± 0.040 b	3.473 ± 0.859 c
orlistat	52.190 ± 2.990 Ab	9.747 ± 1.097 a	0.374 ± 0.006 a	3.093 ± 1.295 c
High-fat diet, beetroot juice, and orlistat	52.190 ± 2.990 Ab	9.747 ± 1.097 a	0.374 ± 0.006 a	3.093 ± 1.295 c
P-Value	0.0007 **	0.0003 **	0.0006 **	0.0006 **
-Value-F	22.62	6.34	13.53	13.61

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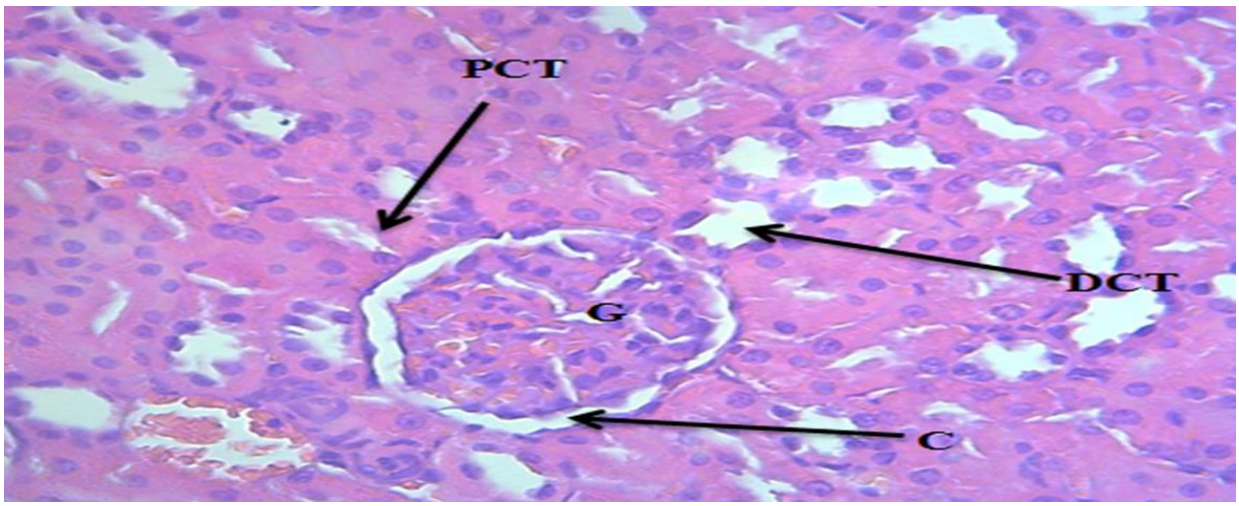
Kidney tissue

The results of the rat kidney tissue, through microscopic examination of the kidney tissue of the group treated with a high-fat diet (A), showed the destruction of the renal glomerulus (DG) .and disintegration of the visceral tissue around the blood vessel (DP) with hemorrhage within the renal tissue (H) and thickening of the vessel wall ,The kidney tissue of the group treated with beetroot juice (B), and the group treated with high-fat food and beetroot juice (C), showedAnd the group treated with high fat food, beet juice and orlistat drug (D) shows the renal glomerulus (G), the proximal convoluted tubule (PCT) and the distal (DCT) and the space around the glomerulus, and the kidney tissue of the group treated with orlistat (E) shows swelling of the renal glomerulus (SG) with Bleeding within the kidney tissue (H) and narrowing of the space around the glomerulus,The kidney tissue of the group treated with high-fat diet and Orlistat (F) showed the renal glomerulus (G), swelling of the cells lining the proximal convoluted tubule (SW), degeneration of the cells lining the distal tubules (D), with hemorrhage within the kidney tissue, and the kidney tissue in the group treated with diet showed High lipids and orlistat show epithelial renal tubule (UT) epithelial hemorrhage within the renal tissue.



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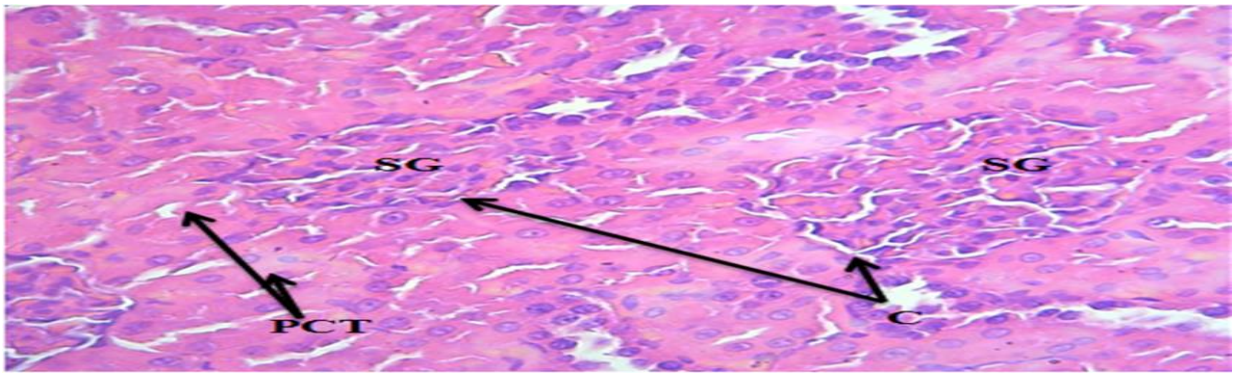

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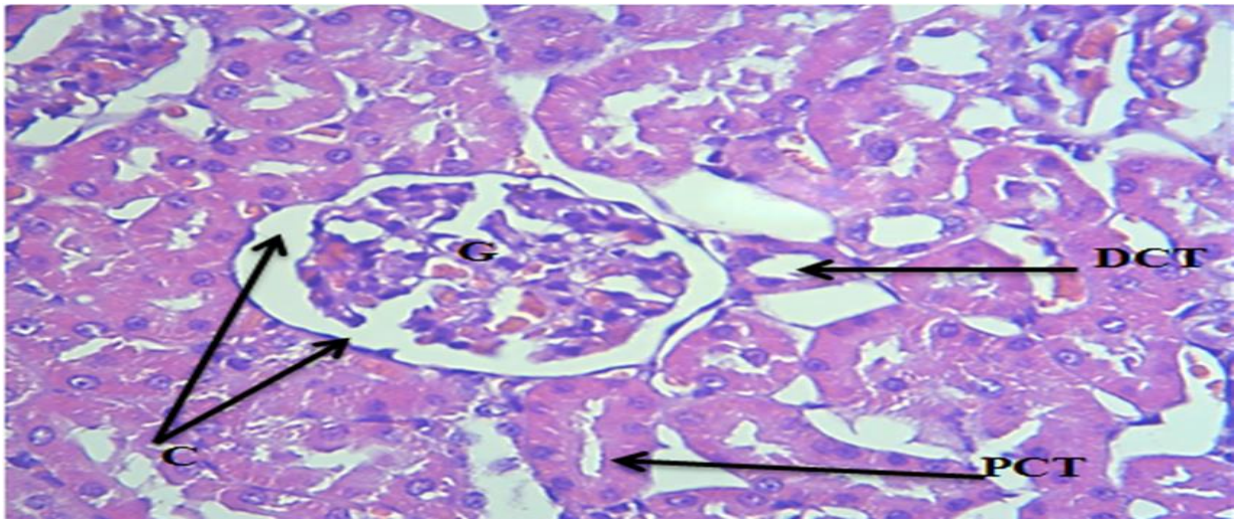

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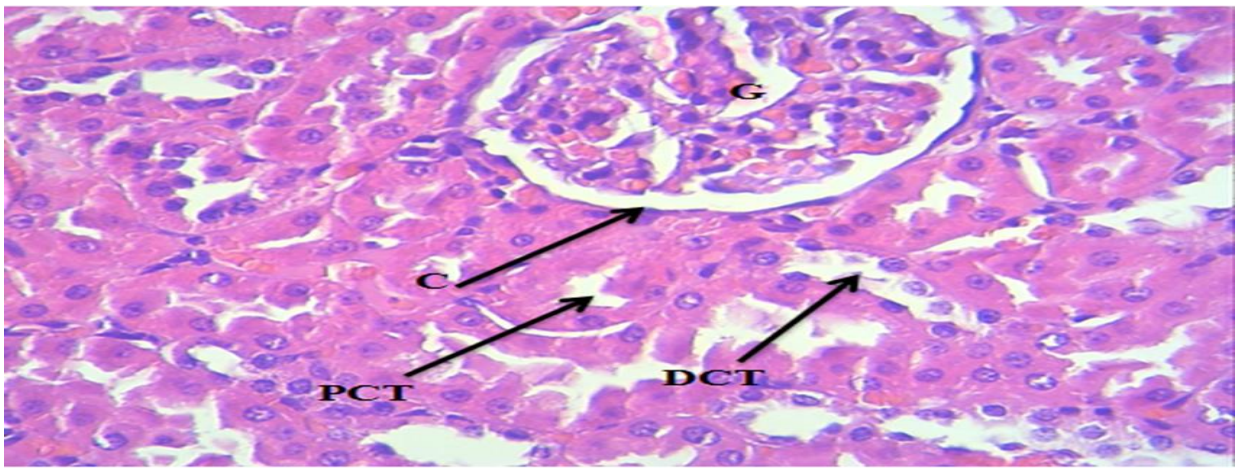

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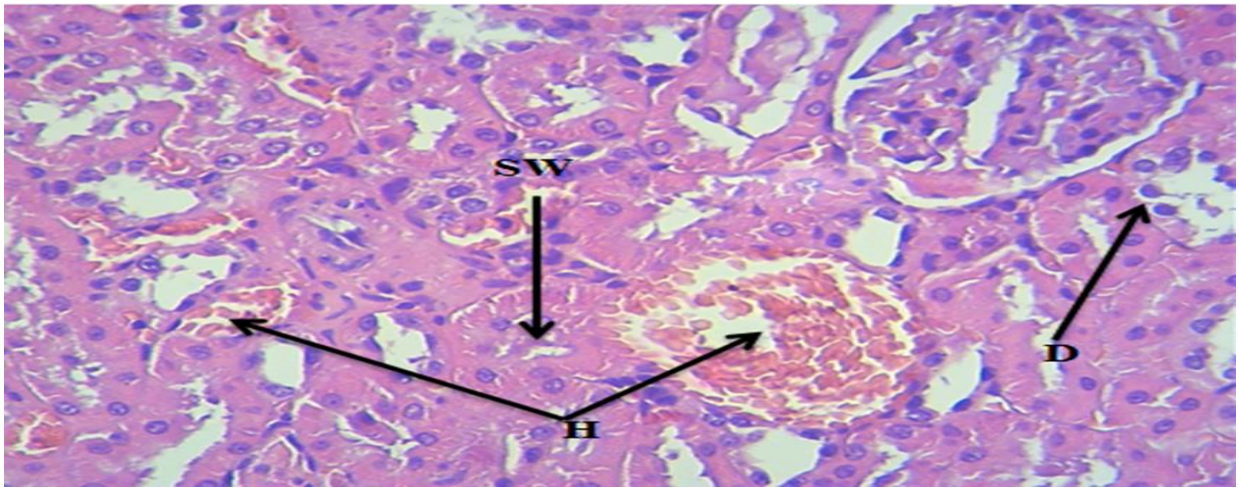

(F)

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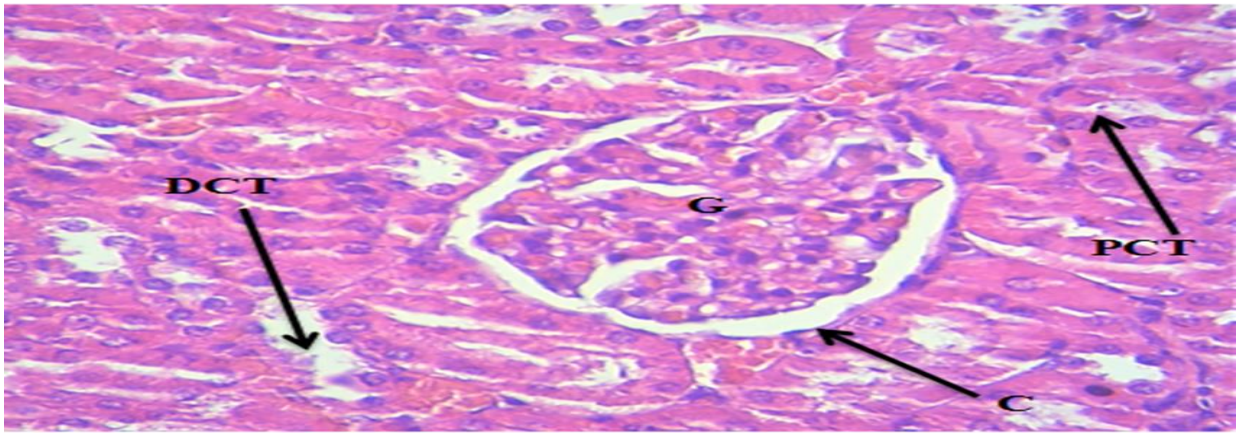

(D)

Discussion

The results of Roche et al. (2009) agreed with the results shown in Table (1), where they showed that the group fed a high-fat diet showed a significant increase in urea and creatinine, and this is due to the significant damage that occurred due to excess fat.It has also been observed through studies that excess fat accelerates the development and emergence of kidney disease. Desnick et al. (2018) because excess cholesterol leads to fibrosis of the interstitial tissues of the kidney, tubule atrophy, and renal ischemia. Extra iron causes increased oxidation and thus leads to an increase in free radicals, as excess iron is a toxic condition that will lead to tissue damage, leading to the formation of molecules (ROS) such as superoxide ions (O2-), hydroxyl radicals (OH), monooxygenase and peroxide Hydrogen (H2O2), which all lead to lipid peroxidation and this leads to oxidative stress that destroys kidney nephrons and thus an increase in the concentration of urea and creatinine in the blood serum (Bhalodia et al. (2010)

Electrolytes are tightly controlled by many hormones through the kidney, which is primarily responsible for preserving and removing electrolytes when necessary and keeping them in a permanent state of balance. Therefore, any damage to the kidney or its microtubules will lead to an imbalance of bodily fluids and their components and lead to Decreased excretion of urea, as it is the basic nitrogenous substance resulting from metabolic waste that is formed and excreted through the urine.As a result of the defect that affects the kidney, this will lead to its aggregation and accumulation in the blood, and thus its levels will rise, i.e. the increase in urea reflects a defect in the filtration function of the kidney. Kang et al. (2014)An increase in body fat percentage and lack of movement and physical activity cause obesity (overweight), and medications and treatments can increase the level of urea in the blood, including diuretics (Sarpal, 2017). The state of increased urea and creatinine also interferes with metabolism and with the regulation of hormones by multiple mechanisms. And the imbalance in the functions of the endocrine glands, such as a high concentration of hormones in the blood and an increase in the effectiveness of the adrenaline gland, and both cases can cause an increase in the concentration of urea and creatinine, and this is consistent with the study of researchers (Fischbach and Warady, 2009). As for the antioxidants, the current results in Table (4-4) agree with the results of the researcher Alipur et al. (2006.(

It was found that the group fed a high-fat diet showed an increase in MDA levels, which is due to a rise in oxidative stress, which generates free radicals that increase lipid oxidation reactions of cell

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membranes and the subsequent partial damage and loss of elasticity through the process of lipid peroxidation.This leads to an increase in MDA, if the unsaturated fatty acids of the cellular membranes are considered the most vulnerable part to the interactions of free radicals because of their possession of double bonds, and the continuation of the generation of free radicals leads to the continuation of the formation of lipid peroxidation, and the lack of antioxidants, whether from external or internal sources, leads to oxidative damage Damage, which is exposed to different tissues of the body, Singh et al. (2014) While the rats fed with high-fat food showed a significant decrease in the levels of GSH, SOD, and CAT, as the results of GSH agreed with (Chen and Li., 2007), which explained the reason for the decrease in GSH as a result of increased consumption of antioxidants to counter the production of excess free radicals in cases of hypercholesterolemia and triglycerides. and generate free radicals also, the reason for the decrease is due to the decrease in the raw materials necessary for its construction, including (nicotine amide diphosphate (NADPH) resulting from the pentaphosphate sugar pathway, which is the catalyst for the action of glutathione reductase, which works to restore the active form of GSH from the inactive form, Perhaps the reason for its low concentration may be due to its increased rate of consumption in the cells of the body because it is one of the most important non-enzymatic antioxidants, as it works to remove free radicals and their products, especially ROS, and the occurrence of oxidative stress that leads to the oxidation of glutathione as a result of its effectiveness as an antioxidant and thus its transformation into the binary oxidized form. GSSG, which is toxic and stimulates the production of new classes of free radicals, also consumes relatively large quantities of glutathione because of its role in restoring the effectiveness of some antioxidants, such as vitamin C, in cases of oxidative stress as a result of its consumption. The level of glutathione can also be affected by other factors such as age, degree of growth, nutritional status, hormonal balance, and the level of the glutathione manufacturing process inside the cell. (Fisher, 2003).

And hyperlipidemia in rats led to an increase in the concentration of MDA and at the same time a decrease in the concentration of GSH compared to the control group, and this is consistent with the results of our current study, and this is also consistent with what the researchers found (Al-Latif and Ali., 2013) and with Stevinkel et al. (2004).In the same context, Alkhamees, (2013) indicated that the high level of triglycerides as a result of oxidative stress and the generation of effective oxygen species resulting from the high concentration of cholesterol led to the inhibition of the triglyceride lipase enzyme responsible for the fragmentation of triglycerides, which leads to an increase in fat metabolism and an increase in its concentrations in Blood serum and liver extract.As for the reason for the decrease in the activity of the SOD enzyme, which is consistent with what was found by Duarte et al. (2010), it is because hyperlipidemia may lead to an increase in blood pressure, and thus the sensitivity to negative feedback that occurs is a reaction in which antioxidant systems increase in response to oxidative stress. Stress and the sensitivity of these systems decrease with the passage of time, which leads to the destruction of organs. This indicates the formation of an excessive amount of ROS from various sources as a result of the oxidative phosphorylation process that occurs in the mitochondria, which causes oxidative damage to the cell with a decrease in the activity of the antioxidant mechanism in both the blood and several other cellular systems that include not only the cells of the vascular wall, but also Those found in blood, Yasunari et al. (2002,(

The reason for the decrease in the activity of SOD in hyperlipidemia patients may be attributed to the fact that the oxidation defense system protects hyperlipidemia patients from the harmful effect of oxidative metabolites, as the SOD enzyme acts as an antioxidant, which plays an important role

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in protecting against the harmful effect of lipid oxidation due to the high levels of super radical formation. Negative oxide, O2.- which works on lipid peroxidation, and this result is consistent with what was reached by the researcher Lto et al. (1995) and Jun et al. (1996), and that the reason for the low level of activity of the catalase enzyme (CAT) may be due to its consumption by oxidation-causing compounds. It destroys cell membranes, the most important of which is MDA, which is present at high levels in the high-fat food group, as the CAT enzyme is one of the removers and scavengers of free radicals, as it removes the effect of (H2O2) toxic in the cell. Bagchi et al. (1993),

In the case of the formation of free radicals and an increase in oxidation, it leads to rapid consumption of defense systems, and a decrease in CAT is accompanied by an increase in nitric oxide (NO), in addition to the inactivation and nitrification of protein by active oxygen species, and this is consistent with what Poulianiti et al. (2016) found. In some chronic clinical cases, the decrease in antioxidants was associated with the inability of the antioxidant systems to compensate for the excessive oxidative stress, which had caused the degradation of proteins including enzymes and cell membranes, which in turn reduced the levels and activity of antioxidant enzymes. Rujito et al. (2015)And that the causative factor responsible for these effects was hydroxylation produced by excess iron, which causes an increase in the production of free radicals, and this is shared with Jankowska et al. (2010). The group fed beetroot juice and the group fed a high-fat diet and beetroot juice showed an increase in the levels of antioxidants MDA, where The results reached by Kanner et al. (2001) are similar to the results of our study. The reason for the high levels of MDA in the group-fed beetroot juice is that it contains compounds that are antioxidants, including betaine. The antioxidant potential of betaine is due to its high electronic donor property and has a major role. Georgiev et al. (2010))

Also, the beet plant contains nitrates that have the ability to interact with and scavenge oxidative stress factors, which may enhance the antioxidant property of red beets (Wink et al. (2001(

It was shown that treating rats on a high-fat diet with beetroot juice from the stems and leaves for 8 weeks reduced oxidative stress in a study similar to ours, Al-Dosari et al. (2011.(

BRJ pretreatment of rats led to a significant increase in SOD and CAT levels. Consumption of this plant root may serve as a tool for strengthening antioxidant defenses to protect cells from oxidative stress and maintain cellular redox balance. Several reports have shown that beetroot contains many antioxidants. El Gamal et al. (2014) Beetroot contains compounds including betalain, isobetalain, vulgaxan thin, neobetanin, p-coumaric acid, and ferulic acid that have strong antioxidant and free radical scavenging effects (Wybraniec 2005; Tesoriere et al. 2004). Betalains as antioxidant and anti-inflammatory agents have a role. Important in releasing NO and lowering blood pressure, Modulation of oxidative stress and inflammation may also be attributed to a regulatory function in the metabolic syndrome such as a blood pressure-lowering effect and most importantly in reducing hyperlipidemia. The effects have been linked to the presence of flavonoids in the juice. Lorizola et al. (2018(

Red beets also contain rutin, epicatechin, and caffeic acid, Georgiev et al. (2010). The results of our study also agreed with the results of the study (Valietal, 2007), which indicated the presence of effective compounds of antioxidants and vital factors in the beetroot plant, including (polyphenols, betaine, and phenols), which indicated that rats fed on beetroot juice contained a high percentage of

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GSH, CAT, and SOD. This is consistent with the study of Hanlon et al. (2011), MDA.While the results differed with the researcher (Raish et al., 2019), who showed that treating rats with red beet extract for 28 days increased GSH, and antioxidant enzymes while feeding red beetroot for 8 weeks reduced SOD, and CAT levels.In another study, the treatment of rats with red beet extract for 60 days resulted in recovery from oxidative stress by reducing MDA malondialdehyde.El-Gammal et al. (2014) The source is confidential. A similar protective effect was observed in rats when treated with red beetroot for 7 days in a similar study. As for the group fed with orlistat, and the group fed with high-fat food and orlistat, its results agreed with the findings of Othman et al. (2019). Orlistat intake with a high-fat diet for a maximum period of eight weeks showed a protective effect on the levels of antioxidants and the increase of the lipid peroxide derivative from the level of MDA in rats.

Effects of orlistat treatment in the orlistat-fed group as well as the high-fat diet and orlistat-fed group on markers of vascular oxidative stress, we determined levels of oxidative stress markers and antioxidants, which include MDA as a marker of oxidative stress, antioxidant enzymatic activities of SOD, CAT, and GSH levels. It was proved that the level of MDA was significantly increased, while the activities of SOD and CAT, in addition to the levels of GSH, decreased significantly in the group fed a high-fat diet, and that the daily treatment of orlistat for obese rats for eight weeks with the high-fat diet,

These changes were significantly reversed. Our findings showed that orlistat treatment significantly improved oxidative stress status in obese rats. This is similar to previous studies, and the improvement in the level of oxidative stress may be attributed to the ability of orlistat to prevent the oxidation of harmful LDL cholesterol, which is pro-atherosclerosis, and this is consistent with (Alagbada, 2016). Oxidative stress and inflammatory progression are interrelated and have been shown to mediate the development of atherosclerotic changes in a hyperlipidemia rat model (Monguchi et al., 2017). The development of atherosclerosis is exacerbated by the frequent and recurrent mechanism of oxidative change and inflammatory processes that transform into a chronic form. Ito et al. (2019).In the case of excessive intake of dietary fat, the lipid components, especially low-density lipoprotein (LDL) cholesterol, are highly permeable to the endothelial layer. Orlistat also performs oxidative modification and converts LDL into an oxidized form, which subsequently leads to an increased appearance of MDA formation. , which prompts more macrophages to engulf excess fat as part of the clearing process. Zakiev et al. (2016).

And the system removes free radicals, which leads to more pathological effects. This is also evaluated by the presence of an advanced oxidation product such as malondialdehyde (MDA). Orlistat acts selectively towards gastrointestinal lipase by preventing the hydrolysis of ingested dietary fat into absorbable free fatty acids and glycerol. For this reason, orlistat is effective in improving antioxidant parameters, white adipocyte volume, and fecal fat excretion in animal models (Othman et al.,2021).

Conclusions

The agreement of beetroot juice in its protective effects in improving the values of the studied variables (biochemical and histological) due to the high-fat diet compared to the drug Orlistat used to treat hyperlipidemia due to having a number of effective compounds to curb the free radicals resulting from the high-fat food.

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