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Effects of Orlistat and herbal mixture extract on renal function and oxidative stress biomarkers in a rat model of high fat diet.

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ABSTRACT

Aims: This study was designed to assess the effectiveness of herbal mixture extracts of pumpkin seed oil, peanuts shell and Orlistat on renal function and oxidative stress biomarkers in male albino rats administrated high fat diet (HFD).

Study design: Fifty male rats were divided into four groups: 1st a normal diet, 2nd HFD, 3rd HFD with Orlistat and 4th HFD with herbal mixture.

Place and Duration of Study: Biochemistry-Chemistry department, Faculty of Science, Beni-Suef University for two years.

Methodology: A group of rats were fed with a standard control diet (1st control group) and another group of rats were fed with a diet containing 35% fat (2nd HFD) for 16 weeks. Then, this group of HFD was divided into 3 groups for the following 6 weeks: 1st group had HFD only, 2nd group had HFD plus 2 mg/kg bw/day Orlistat and 3rd group had HFD plus 5 mg/kg bw/day pumpkins and 2 mg/kg bw/day nutshell extract. Blood and renal tissues were collected for biochemical assays.

Results: HFD group showed a very high significant increase (***P<0.001) in feed intake from low *(216.9 +/- 12.25)* to high *(327.5 +/- 22.00)*, body weight and body mass index. HFD affect the kidney by increasing serum uric acid (**P<0.01) *(1.964 +/- 0.251)* to *(3.106 +/- 0.161)*, urea, creatinine, (***P<0.001) for low density lipoproteins and total cholesterol *(16.71+/-2.27 to 55.78 +/- 4.40 and 70.30 +/- 2.75 to 118.10 +/- 6.35)* respectively, triacylglycerol (**P<0.01) *(54.60 +/- 6.42 to 80.00 +/- 0.65)* and malondialdehyde (***P<0.001) *(35.48 +/- 3.52 to 63.03 +/-1.48)*.These changes improved by the treatments with Orlistat and herbal mixture that decreased the oxidative stress biomarkers.

Conclusion: Rats that fed with HFD showed hypertriglyceridemia, increased oxidative stress and renal alteration. Moreover, suggesting association between lipid peroxidation, obesity and nephropathy, while treatment with Orlistat and herbal mixture ameliorated the harmful effects of the HFD and reduce feed intake.

Keywords: obesity, Orlistat, plant mixture (pumpkin oil with nutshell extract), hyperlipidemia, renal function and oxidative stress.

1. INTRODUCTION

Obesity had been one of the leading public health concerns in industrialized societies for the last 40 years. It had been defined by the WHO as a body mass index or BMI above 30 kg/m^2 [1]. It was reported that obesity increased the risk of end-stage renal disease (ESRD). Excess weight plays a fundamental role in the development of proteinuria and renal damage to patients with severe renal mass reduction [2]. It was found that feeding of HFD that used as inducer for hypercholesterolemia (that made by adding 30% lard or beef tallow and 5% sunflower to the control diet) caused a significant increase in serum MDA level compared with control group. Oxidative stress had been commonly identified in obesity by" the related renal diseases and may be the mechanism underlying the initiation or progression of renal injury in obesity" [3]. The treatment with Garcinia is similar to Orlistat in there action. It significantly decreased MDA level compared with those in group HFD. Garcinia treatment enhanced renal function as a result of HCA-SX derived from Garcinia cambogia which attenuated the increased of oxidative stress biomarkers through reducing lipid peroxidation [4]. On the other hand the treatment with herbal mix showed a decrease in renal function tests and improvement of kidney function.

Pumpkin seed and its oil are rich in unsaturated fatty acid due to high omega -3 fatty acid. Some researches had demonstrated that pumpkin seed oil (PSO) could remarkably reduce bladder pressure, increase bladder compliance and reduce urethral pressure [5]. Peanuts shell contained different bioactive compounds: flavonoids (luteolin and eriodictyol apigenin and chrysin), fatty acids, additional constituents represented by coumarin derivatives (esculetin and daphnetin like compounds) and phenolic acids (coumaric and ferulic acids) [6]. Peanuts shell extract (PSE) showed in vitro inhibitory activity on pancreatic lipase (PL) and lipoprotein lipase (LPL) activity. Also, it was found that PSE had a strong renal protective effect against glycerol-induced acute renal failure (ARF). Pretreatment with PSE in acute renal failure (ARF) rats caused blood urea nitrogen (BUN) and creatinine levels were significantly lower [7]. This study investigates effect of an obesity-associated risk factor on renal oxidative stress, its mechanism of action and biomarkers associated with the effect of Orlistat and herbal mixture on it by the measurement of cholesterol, triglyceride, HDL, LDL, urea, creatinine, uric acid, food consumption, weight weight, body mass index, SOD, MDA and GSH in all four groups of experiments, determine the effect of HFD on these parameters and effect of treatments on it.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 MATERIALS

2.1.1 Diet: Two types of diets had been used, control rat chow diet and special HFD (35 %) for induction of obesity in rats. ***a- Normal rat chow diet:*** It was formed according to Kim et al. [8]. The standard normal rat pellet chow consists of concentrate (350 g), corn (600 g), calcium carbonate, dicalcium phosphate, sodium chloride magnesium oxide and vitamins (50 g). Standard normal rat diet composed of 65% CHO (60% starch+5% sucrose), fat 5%,

crude protein 20%, vitamins and minerals 5%, fibers 5%, metabolic PSE of this diet is 2813 Kcal/Kg with 8% from fat.

b- The HFD: composed of 300 g concentrates, 350 corns, 300 g beef tallow, 50 g vitamins, minerals and fibres according to Kim et al. [8]. Percentage of HFD was 20% crude protein, 35% fat, 40% CHO (starch 35%, 5% sucrose) 5% vitamins, minerals and fibres. Metabolic PSE of this diet is 5130 Kcal/kg, 61% of this PSE from fat. Diet of high fat (HFD) would be (lard and sunflower oil) and starch for induction of obesity from local market by adding 30% lard or beef tallow and 5% sunflower to the control diet [8].

2.1.2 Chemicals: Orlistat is a white to off-white crystalline powder. Orlistat is practically insoluble in water, freely soluble in chloroform, and soluble in methanol and ethanol. It is available for oral administration as a dark-blue or turquoise hard-gelatin capsule. Each capsule contains a pellet formulation consisting of 120 mg of the active ingredient, Orlistat, as well as the inactive ingredients microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, povidone, and talc [9]. Orlistat is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl] methyl] dodecyl ester. Its empirical formula is $C_{29}H_{53}NO_5$, and its molecular weight is 495.7. It was given orally by oral gavage with a dose 2 mg /kg bw/day [10].

2.1.3 Herbal mixture: Mixture herbal extract of Arachics Hypogaea nutshell extract + cucurbitamoschata + morus alb [11] from local market.

1- Pumpkin oils (PO) were purchased from El Kaptin pharmaceutical company; it is a liquid was given by oral gavage in dose of 5mg/kg bw/day [12].

2- Ethanolic extract of Nutshell was prepared by soaking 100 g of dry nutshell in 500 ml ethyl alcohol 95% with daily shaking for 5 days and kept in a refrigerator. Ethanol was evaporated using a rotatory evaporator apparatus which was attached with vacuum pump then the extract was used by dose 2 mg/kg bw/day [11]. Both herbal were mixed together (1:1).

2.2 METHODS:

2.2.1 Animals grouping: Our experiment continued for 22 week and divided into two phases (induction period and treatment period).

a- Induction obesity period: It began from 0-16 th week; by feeding rats with HFD. The animals were divided into four groups; control, HFD, HFD with Orlistat and HFD with herbal mix group.

b- Treatment period: It began form 16-22 th week during this period, HFD group was divided into three groups, (HFD) group continued on fat diet, (HFD+Orlistat) treated group maintained on HFD to 6 weeks with administration of Orlistat treatment at dose of 2 mg/Kg/bw rat/day by stomach tube. While other group HFD maintained on HFD to 6 weeks with administration of herbal mix treatment at dose of 5 mg/kg/bw rat/day for PO and 2 mg/kg/bw rat/day for nutshell by stomach tube.

This study was carried out on white male albino rats (wistar rats) weighing about 100 ± 20g which were used as experimental animals. Rats were fed with a standard control diet for 22 weeks. In the present investigation, the rats were obtained from the animal house of Research Institute of Ophthalmology, El Giza, Egypt. The rats were kept under observation for about 7 days before the onset of experiment to exclude any intercurrent infection. During

the period of the research in the laboratory of Biochemistry, Faculty of Science, Beni Suef University, the chosen animals were housed in plastic container at normal atmospheric temperature (25 ºC ± 5). Food and water were consumed adlibitum. Body weights were recorded weekly and food consumption was calculated daily. Fifty male rats were divided into four groups: 1st a normal diet group (12 rats for control negative maintained on standard normal rat chow diet along the all period of experiment 22 weeks), 2nd HFD group (13 rats HFD) act as control positive, 3rd HFD with 2 mg/kg bw /day Orlistat (12 rats for 6 weeks) and 4th HFD with 5 mg/kg bw/day pumpkins and 2 mg/kg bw/day nutshell extract (13 rats for 6 weeks).

Calculation of average food consumption per each rat was recorded daily by subtracting the amount of food remaining in each day from the measured amount of food provided at the previous day [13]. It was known that metabolic energy of standard rat chow and HFD were 2813 and 5130 Kcal/kg respectively, so the average energy intake was calculated by multiplying the average consumed diet by 2.813 and 5.130 respectively [13]. BMI for rats was measured every week and calculated by dividing the body weight in g by the length (nose to base of tail) in cm^2. Also, BW was measured.

2.2.2 Sampling and tissue preparation:

Blood samples were collected from medial canthus of the eye, via glass capillaries at fasting state. The samples were collected in dry glass centrifuge tubes, allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 minutes at room temperature for separation of serum. The clear, non-haemolysed supernatant sera were separated using clean dry disposable plastic syringes and stored at -20℃ for subsequent biochemical measurements.

Tissue sampling: At the end of experiment (22 week), rats were sacrificed by decapitation and abdominal incision was immediately done after taking of blood sample for separation of kidney tissues. Kidney was taken and washed by saline (0.9%), dried by filter paper and weighed 0.5 g of this tissue then underwent homogenization then centrifuged at 3000 rpm for 15 minutes and the supernatant were kept at -20 ºC for the biochemical tissue analysis of SOD, MDA and GSH. For light microscopic study, the specimens were fixed in 10% neutral- buffered formalin, dehydrated through alcohols, cleared in zylene and then embedded in paraffin wax. Sections (5 µm thick) were stained with haematoxylin and eosin [14].

2.3 EXPERIMENTAL STUDIES

2.3.1 Biochemical examination:-

Cholesterol activity in serum was determined according to the method of (CHOD-PAP-Method) Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing Factor (LCF) [15] and HDL-C in serum determined according to the method of Precipitant and Standard [16]. Serum TG was estimated by the method of Fossati and Prencipe [17] and GPO-PAP-Method. LDL-C can be calculated by the equation: LDL-C (mg %) = total Cholesterol – (triglyceride/5) + HDL-C. Urea concentration was determined colorimetrically [18]. Also, Creatinine was determined according to kinetic determination of creatinine without serum deproteinization [19]. Uric acid level [20] and GSH level also, measured according to colorimetric method that based on the reduction of 5,5' dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound [21] but SOD are metalloenzyme that catalyze the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide [22]. MDA concentration was determined by reaction with thiobarbituric

acid (TBA) in acidic medium at temperature of 95 ºC for 30 min to form thiobarbituric acid reactive product [23].

2.3.2 Statistical analysis of the results:

Data were presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer methods for post-hoc analysis. A value of P<.05 was considered statistically significant. Graph Pad Prism 5 software (San Diego, CA, USA) was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Effect of HFD on feed intake comparable to control group:

Data showing the feed intake figures 1 & 2 were significantly increased in rats of HFD during the period of the experiment compared with that of control group. Feeding of rats on HFD showed a very high significant increase (***P<0.001) in feed intake compared to normal ones that feed on normal diet during 0-16th week (induction time for obesity occur).

Table 1. Means of food intake in control and HFD- induced obesity in rats.

Groups	Means of feed intake (g/day/rat)
Control	216.90 ± 12.25 ^{a}
HFD	327.50 ± 22.00 ^{b}

Each value is the mean ± SEM. Means have different superscript letters indicated a significant variations.

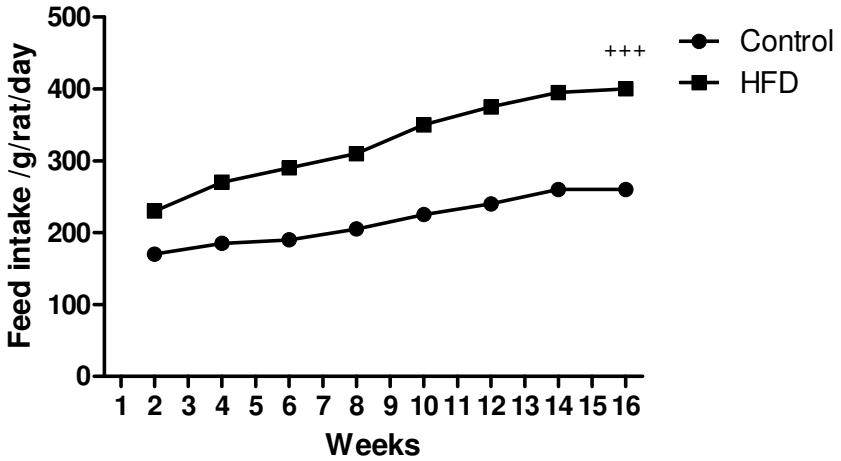

Fig.1 Means of feed intake (g/day/rat) in control and HFD- induced rats (0 to 16th week). Means have a significant variations at $^{+++}$P< 0.001.

3.1.2 Effect of HFD, Orlistat and herbal mix on feed intake:

In this study, feed intake was significantly increased during the period of the experiment in HFD rats compared with that of control group while Orlistat and herbal mix treatment tended to decrease food intake compared with HFD group (Figure 2).

Data shows, in the 18th week HFD group, a very high significant increase (***P<0.001) in feed intake compared to control group, while treatment with Orlistat and herbal mix exerted non-significant decrease in feed intake and weight loss. With continuous feeding of rats on HFD and treatment with Orlistat and herbal mix, continuous increasing had occurred (***P<0.001) in feed intake with these rats that feed on HFD than control group. Also, a very high significant decrease (***P<0.001) in feed intake with these rats that treated with Orlistat and herbal mix and this occurred in 20 week. At the end of the experimental study that took 22 week, we observed a very high significant increase (***P<0.001) in feed intake with HFD group compared to control group, while treatment with Orlistat and herbal mixture showed a very high significant decrease (***P<0.001) in feed intake.

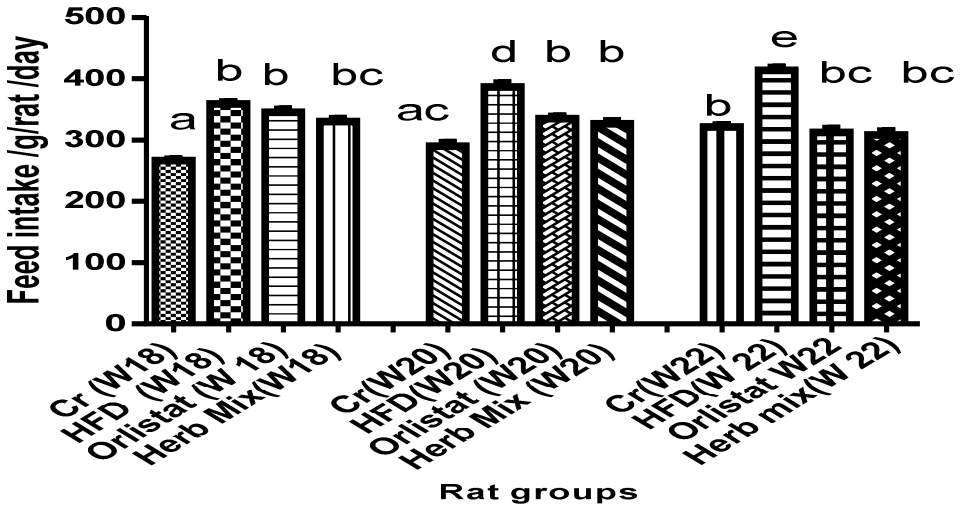

Fig.2 Means of feed intake (g/day/rat) in different groups of experiment (18th-22 week) in rats. *Means have different letters indicated significant variations.*

3.1.3 Effect of diet induced obesity and treatment on body weight and body mass index (BMI):

Our data shows that there was a higher significant in body weight during free diet of HFD compared to control diet. It was found that HFD caused a high significant increase (***P<0.001) in body weight and body mass index from 0-16th to 18-22 wk. At the beginning of the experiments (initial weight) all weight of two groups was the same, control & HFD, and showed non-significant between them in weight. However these weights changed quickly when we feed one of them on HFD and the other was fed on normal diet. We observed that the rats that were fed on HFD had increased in body weight and body mass index compared to normal diet (control rats). These changes occurred 0-16th week and continued to the final 18-22 weeks, (Figure 3 and 4). But these increases in BW and BMI were changed by decreasing it with treatments of HFD with Orlistat and herbal mixture. When high fat diet treated with Orlistat and herbal mix from 18-22 weeks a significant (*P<0.05) and a very high

significant decrease (***P<0.001) occurred in these parameters respectively according to these figures. After the induction period of obesity finished (0-16 week), HFD began treated with Orlistat and herbal mix in 18-22 weeks. We observed that HFD had a very high significant increase (***P<0.001) in body weight and body mass index till the end of experiments in 16-22 weeks compared to control group. However when treatment started in 18-22 weeks, our results showed that the treatment of HFD with Orlistat and herbal mix exerted a significant decrease in BW and BMI as shown in figure 3 and 4.

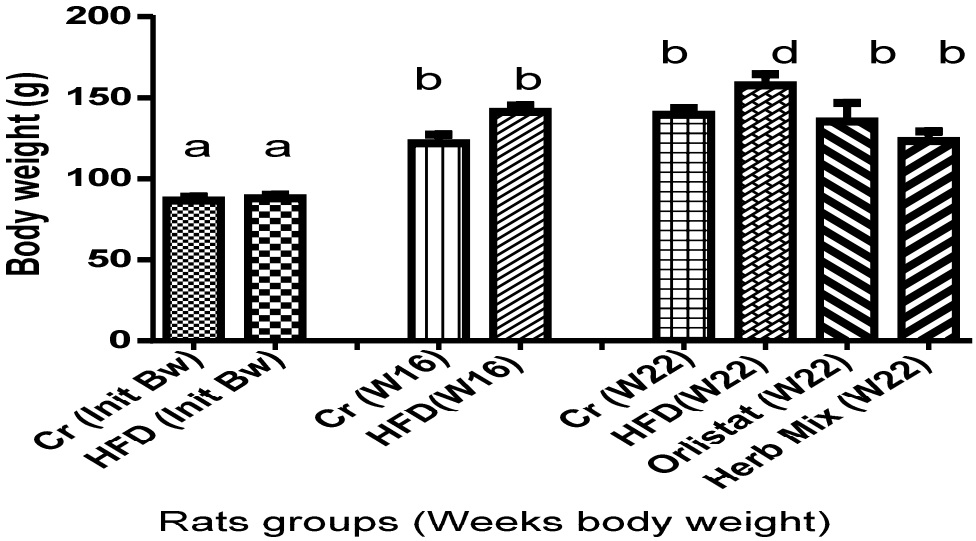

Fig.3 Means of BW in rats during all period of experiment (22 weeks). *Means have different letters indicated significant variations. Data expressed as mean ± SEM.*

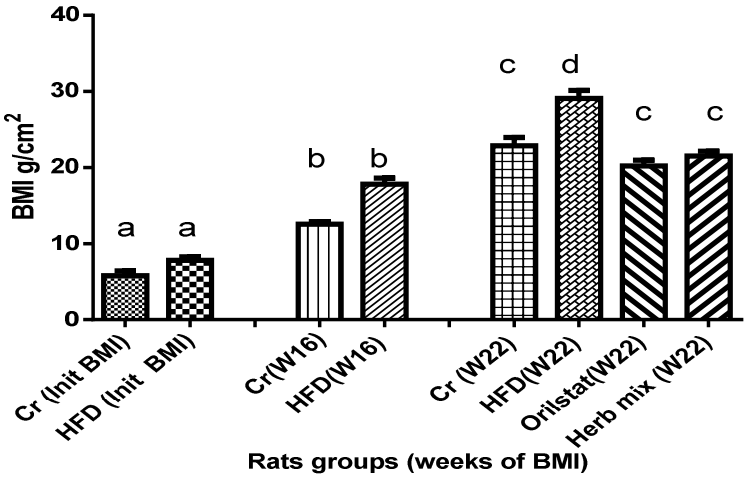

Fig.4 Means of BMI in rats during the period of the experiment (22 weeks). *Means have different letters indicated significant variations. Data expressed as mean ± SEM.*

3.1.4 Effect of HFD, Orlistat and herbal treatment on serum cholesterol, TG, LDL and HDL in rats:

Table 2 manifests the effect of HFD, Orlistat and herbal mix on serum cholesterol, triglyceride, LDL and HDL of HFD rats. HFD rats recorded a very high significant increase (***P<0.001) in serum cholesterol, LDL. They also recorded a high significant increase (**P<0.01) in serum triglyceride but a non-significant decrease of serum HDL as compared to normal rats. While the treatment of HFD with Orlistat recorded a very high significant decrease (***P<0.001) in serum cholesterol and a significant decrease (*P<0.05) in serum triglyceride. Also, the treatment of HFD with herbal mix extract showed a significant decrease (*P<0.05) in serum cholesterol and triglyceride but two treatments for HDL and LDL showed a significant increase (*P<0.05) of serum HDL and a very high significant decrease (***P<0.001) of serum LDL when compared to HFD as shown in table 2.

Table 2. Effect of HFD, Orlistat and herbal treatment on serum cholesterol, TG, LDL and HDL in rats:

Groups	TG (mg%)	Cholesterol (mg%)	LDL (mg%)	HDL (mg%)
Control	54.60±6.42[a]	70.30±2.75[a]	16.71±2.27[a]	40.97±2.35[a]
HFD	80.00±0.65[b]	118.10±6.35[b]	55.78±4.40[b]	44.39±6.75[a]
Orlistat	60.22±6.09[a]	82.78±6.75[a]	23.34±3.48[a]	63.90±4.26[b]
Herbal Mix	59.56±4.55[a]	94.56±5.49[c]	29.66±3.95[a]	60.69±7.15[b]

Data is expressed as mean ± SEM. Identical letters indicates non-significance between groups, while different letters indicate significance variations.

3.1.5 Effect of HFD, Orlistat and herbal treatment on kidney function tests:

Table 3 manifests the effect of HFD, Orlistat and herbal mix on urea, creatinine and uric acid of HFD rats. The HFD rats exhibited no significant increase of urea and a very high significant increase (**P<0.01) of creatinine and uric acid as compared to normal rats. While the treatment of HFD with Orlistat and herbal mix exerted also a non-significant decrease of urea and a significant decrease (*P<0.05) in creatinine. In addition, they recorded a non-significant decrease uric acid when compared to HFD rats as shown in table 3.

Table 3. Effect of HFD, Orlistat and herbal treatment on kidney function tests:

Groups	Urea (mg%)	Creatinine (mg%)	Uric acid (mg%)
Control	24.53 ± 1.02[a]	0.54 ± 0.04[a]	1.96 ± 0.25[a]
HFD	26.22 ± 1.55[a]	0.68 ± 0.03[b]	3.11 ± 0.16[b]
Orlistat	23.19 ± 1.57[a]	0.57 ± 0.02[a]	2.86 ± 0.23[b]

Herbal Mix	23.81 ± 1.25[a]	0.56 ± 0.02[a]	2.86 ± 0.15[b]

Data is expressed as mean ± SEM. Identical letters indicate non-significance between groups, while different letters indicate significance variations between groups.

3.1.6 Effect of HFD, Orlistat and herbal treatment on renal oxidative stress:

Table 4 manifests the effect of HFD, Orlistat and herbal mix on renal oxidative stress of HFD rats. HFD rats exhibited a significant decrease (*P<0.05) of renal GSH and a very high significant decrease (***P<0.001) of renal SOD. They also exhibited a very high significant increase (***P<0.001) of renal MDA as compared to normal rats. While the treatment of HFD with Orlistat exerted a significant increase (*P<0.05) in renal GSH and SOD but a high significant decrease (**P<0.01) in renal MDA as compared to HFD rats. In addition, the treatment of HFD with herbal mix exerted a very high significant increase (***P<0.001) in renal GSH, a significant increase (*P<0.05) in renal SOD and a non-significant decrease in renal MDA when compared to HFD as shown in table 4.

Table 4. Effect of HFD, Orlistat and herbal treatment on renal oxidative stress biomarkers:

Groups	Renal GSH (mg/dl)	Renal MDA (nmol/ml)	Renal SOD (u/gm)
Control	9.31 ± 0.21[a]	35.48 ± 3.52 [a]	548.60 ± 24.10[a]
HFD	7.74 ± 0.25[b]	63.03 ± 1.48[b]	375.60 ± 29.70[b]
Orlistat	9.37 ± 0.22[a]	50.33 ± 1.60[c]	499.80 ± 27.60[a]
Herbal Mix	10.42 ± 0.52[a]	60.17 ± 0.02[b]	462.0 ± 30.70[a]

Data is expressed as mean ± SEM. Identical letters indicates non-significance between groups, while different letters indicated significance variations between groups.

Light Microscopic Examination of the Kidney:

Group (1) the kidney of control rats:

A histological examination of the normal kidney showed that it consists of an outer cortex an inner medulla. The kidney cortex revealed the typical histological structure of the renal corpuscle and the renal tubules. The renal corpuscle includes the glomerulus which consists of small tuft of fenestrated capillaries covered by thin diaphragm and Bowman's capsule fig. 5 a&b. The capsule consists of an inner visceral layer and an outer parietal layer. Also, the renal tubules consist of proximal convoluted tubules which are lined with a brush border cuboidal epithelium. While the distal convoluted tubules are lined with low and non-brush border cuboidal epithelial cells.

Group (2) rats treated with HFD:

Light microscopic finding of kidney from HFD fed rats are summarized as follows: dilated of blood vessels fig. 5c in addition to perivascular and periglomerular oedema infiltrated with a number of inflammatory cells was observed fig. 5d. Also, degenerative change of renal tubules, vacuolated glomerular tuft surrounded with oedema and necrotic cells were noticed fig. 5e and enlargement of Bowman's space fig. 5f.

Group (3) rats treated with HFD with Orlistat:

The present light microscopical observations clearly demonestrated that administration of the Orlistat to an adult HFD rat causes various histological alternation in the kidney tissue. These changes include: (a)-hyperemic glomerular tuft associated with swelling in the lining epithelium of renal tubules and (b)-vacuolar glomerular tuft with swollen endothelial cell lining the Bowman's capsule fig. 6g. The examined kidneys showed blood vessel hypermic, necrotic cells and intertubular hemorrhage fig. 6h&i. While other findings demonstrate a severely congested blood vessels and a focal necrotic area with inflammatory cells infiltration fig. 6j.

Group (4) rats treated with HFD with PO and nutshell extract:

Animals treated with HFD plus PO and nutshell extract showed a histological picture more or less similar to controlled group fig. 6k. While others showed some hemorrhage between renal tubules fig. 6l.

Figure 5: Photomicrograph of the kidneys sections of normal rats (a, b) and treated with HFD (c, d, e and f):

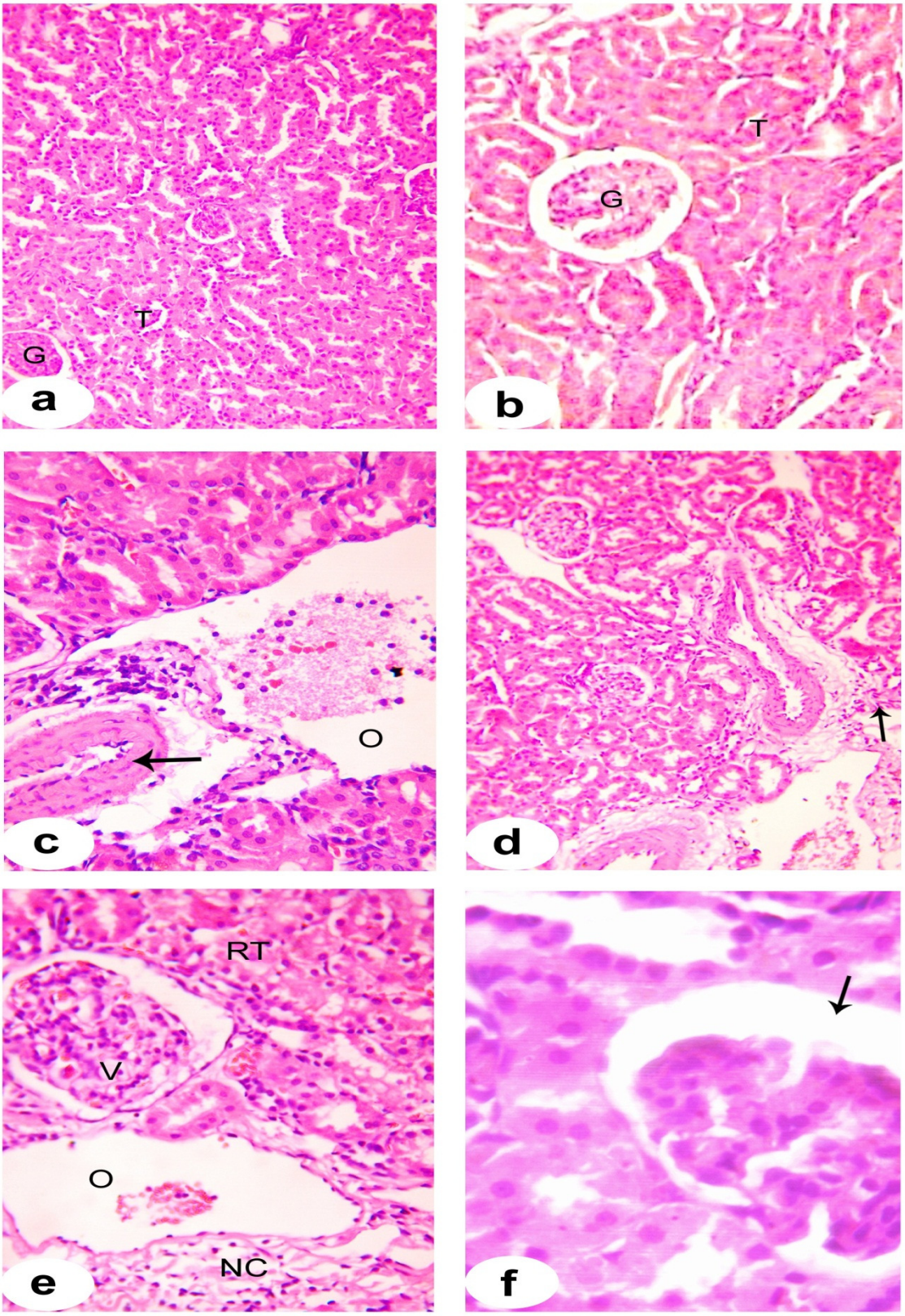
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Fig (5 a&b): Photomicrograph of the kidneys' sections of normal rats showing the glomerulus (G) and renal tubules (t). Fig (5a) H&E x 100 and Fig (5b) H&E x 400.

Fig (5 c): Photomicrograph of the kidneys' sections of rats treated with HFD showing a dilated blood vessel (↑), perivascular oedema (O) and inflammatory cells (▲) infiltration. H & E x 400.

Fig (5 d): Photomicrograph of the kidneys' sections of rats treated with HFD showing perivascular and periglomerular oedema infiltrated with a number of inflammatory cells (↑). H & E x 400.

Fig (5 e): Photomicrograph of the kidneys' sections of rats treated with HFD showing degenerative change of renal tubules (RT), a vacuolated glomerular tuft (v) surrounded with oedema (O) and necrotic cells (Nc). H & E x 400.

Fig (5 f): Photomicrograph of the kidneys' sections of rats treated with HFD showing expansion of Bowman's capsule (↑). H & E x 400.

Figure 6: Photomicrograph of the kidneys sections of rats treated with HFD plus Orlistat (g, H, I, j) and herbal extract (k, l):

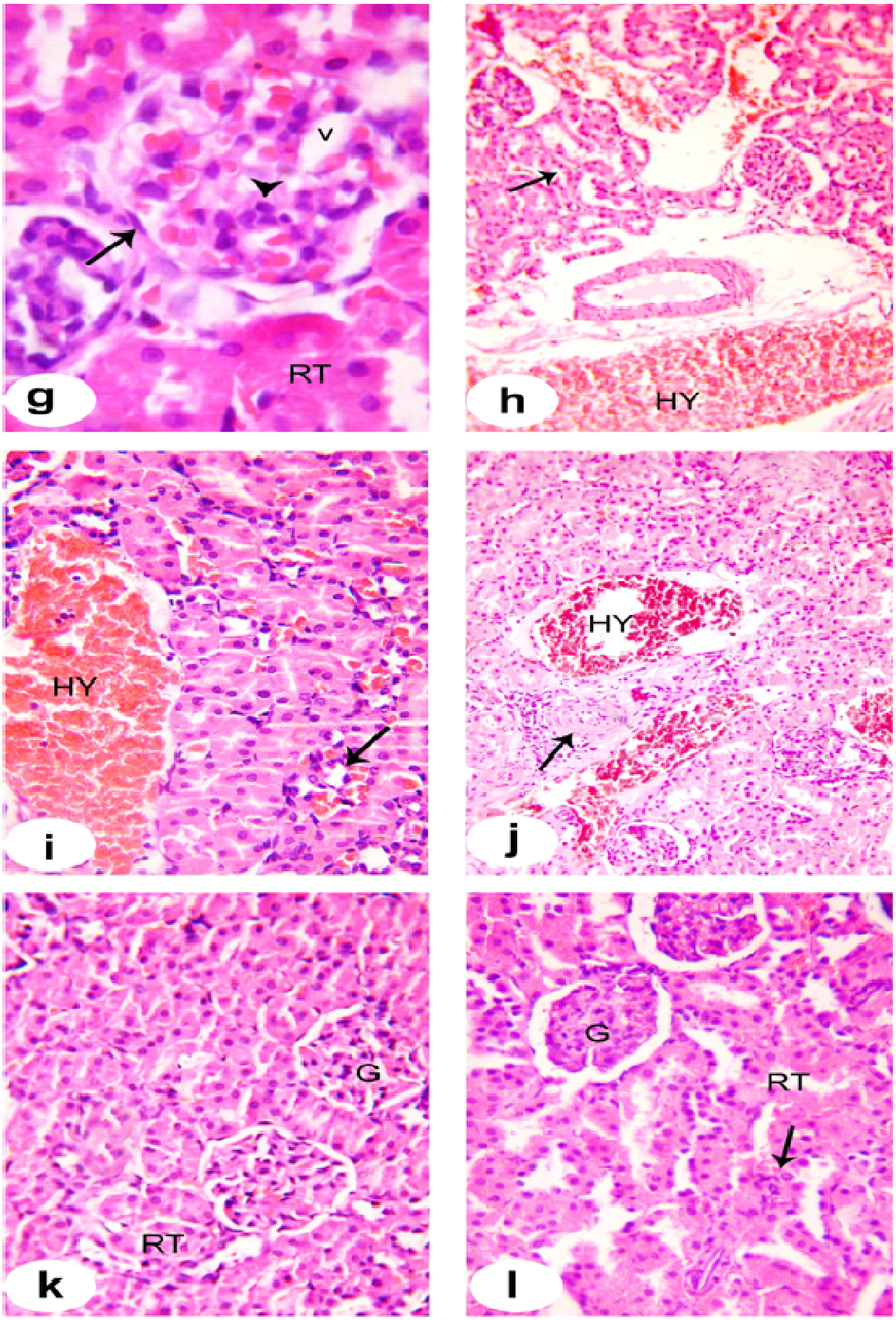
v
RT
g
HY
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Fig (6 g): Photomicrograph of the kidneys' sections of rats treated with HFD plus Orlistat showing hypermic glomerular tuft (▲), swelling in lining epithelium of renal tubules (RT) and vacuolar glomerular tuft (V) with swollen endothelial cells lining the Bowman's capsule (↑). H & E x 400.

Fig (6 h): Photomicrograph of the kidneys' sections of rats treated with HFD plus Orlistat showing blood vessel hypermic with blood (Hy) and necrotic cells (↑).H & E x 400.

Fig (6 i): Photomicrograph of the kidneys' sections of rats treated with HFD plus Orlistat showing severely congested blood vessels (Hy) and intertubular hemorrhage (↑).H & E x 400.

Fig (6 j): Photomicrograph of the kidneys' sections of rats treated with HFD plus Orlistat showing focal necrotic area (↑) with inflammatory cells infiltration and congested blood vessels (Hy). H & E x 200.

Fig (6 k): Photomicrograph of the kidneys' sections of rats treated with HFD plus PO and nutshell extract showing nearly normal structure of glomerulus (G) and renal tubules (Rt). H & E x 400.

Fig (6 l): Photomicrograph of the kidneys' sections of rats treated with HFD plus PO and nutshell extract showing some hemorrhage between renal tubules (↑) and nearly normal structure of glomerulus (G) and renal tubules (Rt). H & E x 400.

3.2 DISCUSSION

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems [24]. It increases the probability of being infected with various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer and osteoarthritis. Newer researches point to obesity as an important risk factor for chronic kidney diseases (CKDs) [24]. Weisinger et al. [25] reported that massive obese patients developed nephrotic range proteinuria. Obesity that is related to glomerulopathy (ORG) is considered an increasing cause of end-stage renal disease (ESRD). Potential mechanisms by which obesity affects renal physiology include: altered renal hemodynamics, insulin resistance, hyperlipidemia, activation of reninangiotensin aldosterone system (RAAS), inflammation and oxidative stress. Also, increases in both glomerular filtration rate (GFR) and renal plasma flow (RPF) are observed in both obese subjects and animals [26].

It is clear in this study that there is a high significant increase of food intake in free diet of HFD during 0th-16th week and 18th-22th weeks compared to the controlled diet. This is may be due to HFD causing hyperphagia which similar to human cafeteria diet. The mechanisms for how saturated fat based beverages contribute to human obesity are clear in rats on an HF choice diet, plasma leptin concentrations and proopiomelanocortin mRNA increased and neuropeptide Y mRNA decreased [27]. Both palatability and PSE density contribute to fat hyperphagia and reduced satiation signaling accompanying HFD consumption which can contribute to overconsumption and often lead to obesity. The current data shows a significant increase in body weight and BMI especially in the 16th week of HFD in accordance with Akiyama et al. [28]. Increased BW and BMI may occur due to the increase of caloric intake resulting in more adipose tissue deposition than starch diet.

When we treated HFD with Orlistat and herbal mix ameliorated feed intake, significantly decreased BW and BMI. These effects of Orlistat are due to its blocking to the absorption of

fat by inhibiting gastric and pancreatic lipase enzymes leading to the increased excretion of fat in faeces [29]. Also, treatment with PO has its effects on feed intake, weight gain and body mass index. This is because it contains a number of anti-oxidants and an excellent source of protein, zinc, magnesium, manganese and phosphorus. They also contain a high amount of tryptophan, an essential amino acid involved in the synthesis of a key brain chemical called serotonin which is involved in mood, sleep and appetite regulation [30]. On the other hand the PSE were screened for inhibitory effects on pancreatic lipase (PL) and lipoprotein lipase (LPL) activities as well as on lipolysis of 3T3-L1 adipocytes. It was found that PSE could prevent the body weight gain induced by feeding a HFD to male Wistar rats with 1% of PSE [11].

Our study indicated that HFD induced obesity may leads to morbidity as well as body biochemical changes. There is usually increased level of total cholesterol, LDL-C, triglycerides and decreased level of HDL-C. These results are in accordance with those of Szczygielska et al. [31]. Mean total cholesterol and triglycerides concentrations were higher in obese persons compared with normal weight people and HDL concentration was lower in obese people compared to normal and overweight individuals [31]. While treatment of HFD with Orlistat showed that patients receiving the two higher doses of Orlistat achieved significantly reduced levels of total cholesterol and LDL-C. LDL/HDL ratio was significantly reduced in the highest dose of Orlistat group compared to placebo [32] due to inhibition of gastric and pancreatic lipases. It inhibits the hydrolysis of dietary triglycerides, consequently limiting the absorption of monoglycerides and free fatty acids. Also, the treatment of obesity in rats with PO and nutshell produced a significant decrease in serum cholesterol, serum triglyceride and a high significant decrease in serum LDL while a very high significant increase in serum HDL as compared to the HFD rats. These results are agree with that of Moreno et al. [11] and Gossell-Williams et al. [33] who found that the consumption of pumpkin seeds and nutshell extract is also beneficial in regulating serum lipid levels.

A study revealed that a diet supplemented with PO provided lower total cholesterol, lower LDL-C, lower triglycerides and higher HDL-C levels. PO may reduce LDL-C. The oil may also lower cystolic and diastolic blood pressures due to its high concentration of a phytoestrogens [33], which reduce serum cholesterol levels by the inhibition of cholesterol absorption and hepatic cholesterol esterase. On the other hand, it had been shown to lower LDL-C equivalently in hypercholesterolemic persons by suppressing cholesterol absorption. PO has a high content of linoleic acid (42.6%). PO has a good amount of oleic acid (35.3%). Oleic acid (monounsaturated fatty acid) was reported to reduce total plasma cholesterol and LDL-C. Belonging to the reduction of the isoprenaline-stimulated lipolysis by PSE suggests that PSE may be taken inside the cells, Moreno et al. [34], where it decreases the activity of HSL. Thus, the inhibition of HSL may reduce levels of circulating (FFA) linked to insulin resistance in obese patients. BW and body weight gain, were significantly lower in rats fed the high-fat diet with 1% of PSE than in those fed with the HFD alone. The rats treated with PSE showed reduced triacylglycerol content in the liver. The inhibitory activity of PSE on the lipid metabolic enzymes and the increase in fecal fat excretion suggests that PSE might be useful as a treatment to reduce the dietary fat absorption. The observed reduction in intracellular lipolytic activity of cultured 3T3-L1 adipocytes may reduce the levels of circulating free fatty acids and the reduction of the adipocyte lipolysis.

HFD caused a significant elevation in serum creatinine (table 3); a specific indicator of glomerular function and urea level owing to consumption of HFD which result in metabolic syndrome marked by obesity, hyperlipidemia and associated with oxidative stress which leading to renal dysfunction, characterized by high level of creatinine and blood urea nitrogen. In obese people, increased serum creatinine was observed, suggesting that obesity caused elevation in renal function test and produced proteinuria, concomitantly with

other risk factors such as hypertension, diabetes and dyslipidemia [35]. Renal triglyceride accumulated in obese rats accompanied by hypoalbuminemia and elevated blood urea nitrogen. Visceral and renal fat accumulation through consumption of a HFD leads to marked renal sympathetic activation, which is related to increased responsiveness to central sympathoexcitatory effects of leptin that contributes to the development of hypertension [36]. Obesity had been associated with elevated serum uric acid. Increases in serum uric acid concentration showed positive association with BMI, WHR, Waist/thigh girth and sub-scapula triceps skin fold ratios. The risk of gout was increased among men who had been overweight in adolescence [37]. While treatment with Orlistat in our data showed that improvement in the level of renal function tests and decrease in kidney disease which found that the mechanism of action of gastrointestinal lipase inhibitors, the temporal association of accelerated renal function decline with the commencement of Orlistat. Serum uric acid, urea and creatinine levels for Orlistat – treated obese patients were decreased [38] it was reported that Orlistat treatment was beneficial for patients to get over obesity because it improved metabolic processes and therefore kidney and liver functions.

Also, treatment with herbal mix (PO) showed that decrease in renal function tests and improvement of kidney function [39]. It was observed that administration of flax/pumpkin or purslane/pumpkin seed mixtures showed an announced significant decrease in the levels of serum urea and creatinine as compared with hypercholesterolemic control group (purslane or flax seeds were used as sources of ω-3 fatty acids). Flax seed is the richest natural source of plant lignans, which are PAF receptor antagonists. Platelet activating factor plays a key role in the mediation of inflammation, mitogenesis and alteration of glomerular permselectivity. Flaxseed could potentially inhibit various mechanisms associated with the progression of renal diseases. Flax and pumpkin seeds mixtures are rich in PUFAs and antioxidant compounds in animals. Pumpkin seeds appear to reduce levels of substances that promote stone formation in the urine. Some researches had demonstrated that PSO could remarkably reduce bladder pressure, increase bladder compliance and reduce urethral pressure [5].

On the other hand treatment with PSE caused reduction in values of urea nitrogen and creatinine due to the active component luteolin that found in it [40]. There was a concomitant significant reduction in the elevated of marker parameters of kidney toxicity BUN and serum creatinine in luteolin pretreatment groups. This showed the efficacy of luteolin in regulating renal functions [40]. Luteolin increases the level of heme oxygenase-1 protein (HO-1) which is the inducible isoform of the HO system. It is a rate limiting enzyme converting heme into equimolar amounts of iron, carbon monoxide and biliverdin. HO-1 is thought to have antioxidant and cytoprotective roles. It was suggested that one of the mechanism of the renoprotective effect of luteolin may be related to increasing HO-1 expression and elevating antioxidant in diabetic nephropathy. PSE significantly reduced renal oxidative damage [7] and had a strong renal protective effect against glycerol-induced acute renal failure (ARF).

Dietary HFD caused a significant decrease of kidney GSH, kidney SOD and a high significant increase of kidney MDA as compared to the normal rats. Oxidative stress is caused by an imbalance between increased production of ROS and/or reduced antioxidant activity, leading to oxidative damage to cells or tissue including lipids, proteins and DNA. Studies had suggested that obesity is associated with increased oxidative stress. Oxidative stress had been commonly identified in obesity-related renal diseases and may be the mechanism underlying the initiation or progression of renal injury in obesity [41]. ROS are highly reactive molecules that oxidize lipids and proteins, cause cellular injury and promote glomerular and renal tubule injury and associated proteinuria. HFD induced obesity is accompanied by increased renal tissues oxidative stress, which is characterized by

503 reduction in the antioxidant enzymes activities and glutathione levels, which correlate with
504 the increase in MDA and protein carbonyl levels in most tissues [42].

505 While our study indicated that treatment of HFD rats with Orlistat produced a significant
506 increase in kidney GSH, SOD and a high significant decrease in kidney MDA as compared
507 to the drug-administrated-control rats. Garcinia, this is drug similar in action to Orlistat in
508 weighting loss significantly decreased levels of serum MDA and renal MDA compared with
509 HFD as result of hydroxy citric acid (HCA-SX) that inhibit ATP citrate lyase which catalyse
510 extra mitochondrial cleavage of citrate to oxaloacetate giving acetyl COA which used in fatty
511 acid synthesis, suggesting that Garcinia has hypolipidemic action and reduces MDA in
512 kidney, so HCA improves lipid peroxidation [4].

513 On the other hand treatment of HFD rats with herbal mix produced a significant increase in
514 kidney GSH, SOD and a high significant decrease in kidney MDA as compared to the HFD
515 rats. Wang et al. [7] reported that pretreatment with PSE in acute renal failure (ARF) rats
516 caused GSH in serum and renal cortical significantly increased. In addition, renal cortex
517 homogenate MDA levels were significantly lower also, the treatment with PO contained
518 omega-3 caused significantly lower for oxidative stress which had increased following
519 reperfusion injury [43]. Preventive oral administration of omega-3 supplement may decrease
520 oxidative stress, kidney dysfunction following reperfusion injury and may result in better anti-
521 oxidation status.

522 **Photomicrograph of the kidneys sections of normal HFD, Orlistat and herbal**
523 **treatment:**

524 Obesity is a chronic metabolic disorder that results from the imbalance between PSE intake
525 and PSE expenditure characterized by enlarged fat mass and elevated lipid concentration in
526 blood. Many attempts had been made to correct the metabolic disparity of the obesity
527 condition, producing a number of reagents including fibrates, sibutramine (an anorectic or
528 appetite suppressant) and Orlistat but no drug is free from severe side effects [44]. The
529 present study demonstrates that a high-fat diet, fed for as little as 0 to 22 wk, figure 5
530 produced significant histologic, biochemical and functional changes in the kidney in
531 comparison with normal group. These changes included expansion of Bowman's capsule.
532 The mechanisms responsible for the enlargement of Bowman's space in obesity are
533 uncertain but may be related to the marked glomerular hyperfiltration; it is interesting that the
534 GFR was increased by almost the same percentage (38%) as the Bowman's space
535 increase. In our histological study, dilatation of glomerular capillaries and large blood vessels
536 had been seen. In addition to tubular deformations, histologically detected glomerular
537 atrophy, necrosis and oedema similar results were reported by Altunkaynak et al. [45]. The
538 increase in the volume of the kidneys of HFD-fed rats may have resulted from oedema due
539 to mononuclear cell infiltrations among the tubules. It is clearly understandable that dilatation
540 may lead to a volumetric increase in the kidney. According to Hvizdos and Markham [46],
541 Orlistat is a novel non-systemic treatment for obesity it inhibits lipases in the gastrointestinal
542 tract, preventing the absorption of approximately 30% of dietary fat. The present study
543 revealed that the administration Orlistat revealed that focal necrotic area with inflammatory
544 cells infiltration and blood vessels show congestion and some renal tubules suffer from
545 degeneration. Similar results were reported by Korzets et al. [47] who found that renal biopsy
546 findings showed that the chronic kidney disease (CKD) was attributable to
547 nephrocalcinosis/oxalate nephropathy, resulting in a chronic tubulo-interstitial nephropathy,
548 while acute tubular necrosis. HFD increase serum uric acid (table 3) that initiates renal urate
549 crystal producing histopathological changes. Orlistat was capable of increasing urinary
550 oxalate levels many-fold, especially in rats fed high fat or oxalate-enriched diet. A rise in the
551 ion-activity product of calcium oxalate was also demonstrated. Interestingly, no renal

depositions of oxalate were seen on examination of renal tissue at the end of the study, a fact probably explained by the relatively short period of time (6 weeks only) that the animals were treated with Orlistat. Also, in 2008, Karamadoukis et al. [48] retrospectively examined more than 800 renal biopsies, looking specifically for the combined presence of acute tubular necrosis and crystal deposition. Due to its high phosphorus content in fluted pumpkin seed oil, it is said to be a potential agent in reducing kidney bladder stone disease. HFD increased uric acid that elevate urinary acidity which increase the renal histological changes while treatment with pumpkin seed oil decreased renal acidity (toward alkalinity) due to its high phosphorus content so renal histological changes had been improved.

4. CONCLUSION

From the above results we concluded that there is a significant relationship between fatty diet intake and structural changes to the kidney with the elevated levels of urea, creatinine, uric acid, TG, LDL, and the renal injuries were associated with increased oxidative stress monitored by increased MDA level whereas GSH and SOD production decreases in addition to a decrease in the numerical density of glomeruli, tubular deformations, prominent dilatation of the renal vessels, tubules, glomerular necrosis and atrophy and basal membrane thickening. While oral administration of Orlistat and herbal mix extracts reduced the level of circulating lipids as well as the decrease of body weights in male albino rats, these extracts improve kidney functions by decreasing oxidative stress (MDA), increasing antioxidant factors (GSH and SOD) and improve the defects that caused by obesity.

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COMPETING INTERESTS

"Authors have declared that no competing interests exist."

AUTHORS' CONTRIBUTIONS

All authors carried out experimental work; biochemical analysis, statistical analysis, interpretation and discussion of the results related to their part of the work.

"Author 1' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author 2' and 'Author 3' designed the study, managed the analysis of the study and parts of histopathology and biochemistry. Author 4' managed the literature searches. All authors read and approved the final manuscript."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

For manuscripts involving animal experiments, "All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Term: Definition for the term

APPENDIX

Term	Definition for the term
ARF	Acute renal failure
BMI	Body mass index
BUN	Blood urea nitrogen
BW	Body weight
CH	Cholesterol
CKD	Chronic kidney disease
Cre	Creatinine
ESRD	End-stage renal disease
FFA	Free fatty acid
FPSO	Fluted pumpkin seed oil
GFR	Glomerular filtration rate
GSH	Reduced glutathion
HCA	Hydroxy citric acid
HDL	High density lipoprotein
HFD	High fat diet
HO-1	Heme oxygenase-1
HSL	Hormone sensitive lipase
LCF	Lipid Clearing Factor
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
MDA	Malondialdehyde

Nc	Necrotic cells
ORG	Obesity-related glomerulopathy
PAF	Platelet-activating factor
PL	Pancreatic lipase
PSE	Peanut shell extract
PSO	Pumpkin seed oil
PUFAs	Polyunsaturated fatty acid
RAAS	Reninangiotensin-aldosterone system
ROS	Reactive oxygen species
RPF	Renal plasma flow
RT	Renal tubules
SOD	Superoxide dismutase
TG	Triglyceride
WHO	World health organization
WHR	Waist hip ratio

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