

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

Kamal A. Amin^{1*}, Sana'a R. Galaly², Wala'a G. Hozayen³ and Shima'a M. Ramadan³.

¹ Biochemistry Department, Faculty of Veterinary medicine, Beni-Suef University.

¹ Dammam University, college of Science, Chemistry Department

² Zoology Department, Histology, Faculty of Science, Beni-Suef University.

³ Biochemistry-Chemistry Department, Faculty of Science, Beni-Suef University.

ABSTRACT

Aims: This study was designed to assess the potency of herbal mixture extracts of pumpkin seed oil and peanuts shell against Orlistat in male albino rats administrated high fat diet.

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

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 high fat diet) for 16 weeks. Then, this group of high fat diet was divided into 3 groups for the following 6 weeks: 1st group had high fat diet only, 2nd group had high fat diet plus 2 mg/kg bw/day Orlistat and 3rd group had high fat diet 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: High fat diet group showed a high significant increase ($P<.001$) 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 ($P<.01$) (1.964 +/- 0.251) to (3.106 +/- 0.161), urea, creatinine, ($P<.001$) 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 ($P<.01$) (54.60 +/- 6.42 to 80.00 +/- 0.65) and malondialdehyde ($P<.001$) (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 high fat diet 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 high fat diet 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/m² [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]. While treatment with Garcinia is similar to Orlistat in 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 (MDA) [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 peanut shell extract had a strong renal protective effect against glycerol-induced acute renal failure (ARF). Pretreatment with peanut shell extract (PSE) in acute renal failure (ARF) rats caused blood urea nitrogen (BUN) and creatinine (Cre) 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 high fat diet 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

64 and vitamins (50 g). Standard normal rat diet composed of 65% CHO (60% starch+5%
65 sucrose), Fat 5%, crude protein 20%, vitamins and minerals 5%, fibers 5%, metabolic
66 energy of this diet is 2813 Kcal/Kg with 8% from fat.

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

73 **2.1.2 Chemicals:** Orlistat is a white to off-white crystalline powder. Orlistat is practically
74 insoluble in water, freely soluble in chloroform, and soluble in methanol and ethanol.
75 XENICAL is available for oral administration as a dark-blue or turquoise hard-gelatin
76 capsule. Each capsule contains a pellet formulation consisting of 120 mg of the active
77 ingredient, Orlistat, as well as the inactive ingredients microcrystalline cellulose, sodium
78 starch glycolate, sodium lauryl sulfate, povidone, and talc [9]. Orlistat is (S)-2-formylamino-4-
79 methyl-pentanoic acid (S)-1-[[[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester. Its
80 empirical formula is $C_{29}H_{53}NO_5$, and its molecular weight is 495.7. It was given orally by oral
81 gavage with a dose 2 mg /kg bw/day [10].

82 **2.1.3 Herbal mixture:** Mixture herbal extract of (Arachis Hypogaea nutshell extract +
83 cucurbitamoschata + morus alb [11] from local market.

84 1- Pumpkin oils were purchased from El Kaptin pharmaceutical company, it is a liquid
85 was given by oral gavage with dose 5mg/kg bw/day [12].

86 2- Ethanolic extract of Nutshell was prepared by soaking 100 g of dry nutshell in 500 ml
87 ethyl alcohol 95% with daily shaking for 5 days and kept in a refrigerator. Ethanol was
88 evaporated using a rotatory evaporator apparatus which was attached with vacuum pump
89 then the extract was used by dose 2 mg/kg bw/day [11]. Both herbal were mixed together
90 (1:1).

91 2.2 METHODS:

92 **2.2.1 Animals grouping:** Our experiment continued for 22 week and divided into two
93 phases (induction period and treatment period).

94 **a- Induction obesity period:** It began from 1st-16th week; by feeding rats with high fat
95 diet (HFD). The animals were divided into four groups; control, HFD, HFD with Orlistat and
96 HFD with herbal mix group.

97 **b- Treatment period:** It began from 16-22th week during this period, HFD group was
98 divided into three groups, (HFD) group continued on fat diet, (HFD+Orlistat) treated group
99 maintained on high fat diet to 6 weeks with administration of Orlistat treatment at dose (2
100 mg/Kg/bw rat/day) by stomach tube. While other group HFD maintained on high fat diet to 6
101 weeks (12 rats) with administration of herbal mix treatment at dose (5 mg/kg/bw rat/day for
102 pumpkin oil and 2 mg/kg/bw rat/day for nutshell) by stomach tube.

103 This study was carried out on white male albino rats (wistar rats) weighing about 100 ± 20 g
104 which were used as experimental animals. Rats were fed with a standard control diet for 22
105 weeks. In the present investigation, the rats were obtained from the animal house of
106 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 ± 5). Food and water were consumed ad libitum. 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 High fat diet 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]. Calculation of energy intake of consumed diet: Metabolic energy of standard rat chow and HFD were 2813 and 5130 Kcal/kg respectively. The average energy intake of consumed diet was calculated by multiplying the average consumed diet by 2.813 and 5.130 respectively according to consumed diet [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, body weight 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°C 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 r.p.m. for 15 minute and the supernatant were kept at -20°C 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 cholesterol in serum determined according to the method of Precipitant and Standard [16]. Serum TG was estimated by Fassati and Prencipe [17] and GPO-PAP-Method. LDL cholesterol can be calculated by the equation: LDL Cholesterol (mg %) = total Cholesterol – (triglyceride/5) + HDL Cholesterol. Urea concentration was determined colorimetrically [18]. Also, determination of Creatinine according to Kinetic Determination of Creatinine without Deproteinization in Human Serum [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

153 [21] but SOD are metalloenzyme that catalyze the dismutation of superoxide anion to
154 molecular oxygen and hydrogen peroxide [22] in addition to, MDA concentration was
155 determined by reaction with Thiobarbituric acid (TBA) in acidic medium at temperature of
156 95°C for 30 min to form thiobarbituric acid reactive product [23].

157 **2.3.2 Statistical analysis of the results:**

158 Data were presented as mean $\pm$ S.E.M and analyzed using one-way analysis of variance
159 (ANOVA) followed by Tukey-Kramer methods for post-hoc analysis. A value of $P < .05$ was
160 considered statistically significant. Graph Pad Prism 5 software (San Diego, CA, USA) was
161 used for statistical analysis.

162

163

164 **3. RESULTS AND DISCUSSION**

165

166 **3.1 RESULTS**

167

168 ***3.1.1 Effect of HFD on feed intake comparable to control group:***

169

170 Data showing the feed intake figures 1 & 2 were significantly increased in rats of HFD during
171 the period of the experiment compared with that of control group. Feeding of rats on high fat
172 diet showed a high significant increase ($P < .001$) in feed intake compared to normal ones
173 that feed on normal diet during 0-16th week (induction time for obesity occur).

174

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

176

Groups	Means of feed intake (g/day/rat)
Control	216.9 $\pm$ 12.25 ^a
HFD	327.5 $\pm$ 22.00 ^b

177

178 Each value is the mean $\pm$ SEM. Means have different superscript letters indicate significant
179 variation at ($P = .05$).

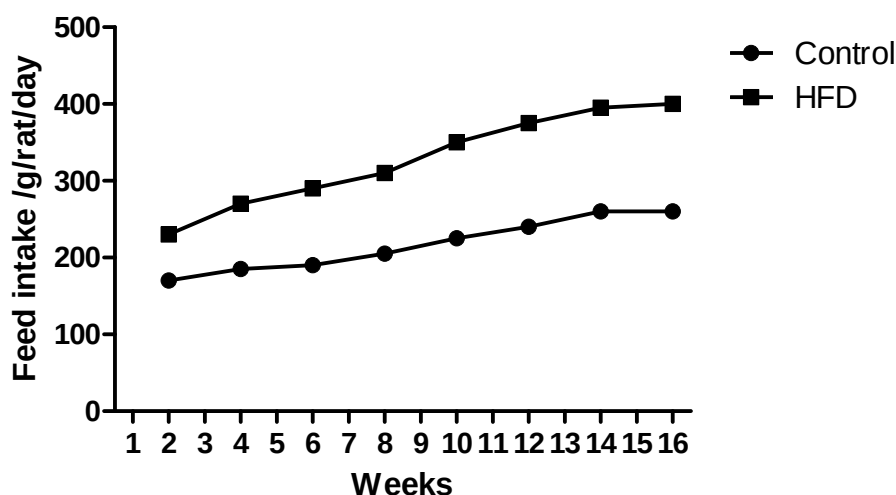


Fig.1 Means of feed intake (g/day/rat) in control and HFD- induced rats (0 to 16th week). Feed intake increased significantly in rats fed on HFD from 0 to 16th week compared with that of control group. Means have different letters indicate significant variation at ($P=0.05$). Data expressed as mean $\pm$ SE with twelve animals in each group.

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.

Data shows, in the 18th week high fat diet group, a 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 high fat diet and treatment with Orlistat and herbal mix, continuous increasing had occurred ($P<0.001$) in feed intake with these rats that feed on high fat diet than control group. Also, a 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 high significant increase ($P<0.001$) in feed intake with high fat diet group compared to control group, while treatment with Orlistat and herbal mixture showed a high significant decrease ($P<0.001$) in feed intake.

214 **Table 2. Effect of HFD, Orlistat and herbal mix on feed intake:**

215

Groups	Weeks	Means of feedintake (g/day/rat)
Control	18 th	266.3 ± 2.3 ^a
HFD	18 th	358.8 ± 3.1 ^b
Orlistat	18 th	345.0 ± 5.0 ^b
Herbal mix	18 th	330.0 ± 5.4 ^{bc}
Control	20	290.0 ± 6.1 ^{ac}
HFD	20	386.3 ± 6.2 ^d
Orlistat	20	335.0 ± 3.5 ^b
Herbal mix	20	326.8 ± 4.5 ^b
Control	22	321.3 ± 3.1 ^b
HFD	22	413.8 ± 4.7 ^e
Orlistat	22	312.5 ± 6.6 ^{bc}
Herbal mix	22	308.0 ± 6.3 ^{bc}

216

217 Each value is the mean ± SEM. Means have different superscript letters indicate significant
 218 variation at ($P=0.05$).

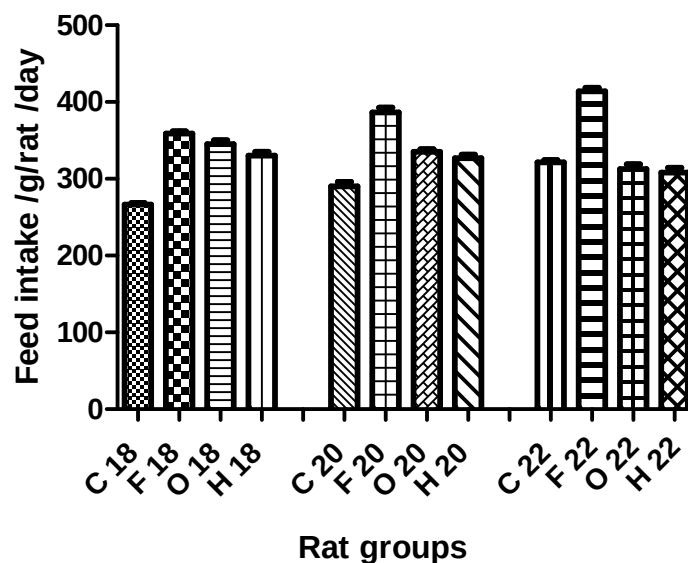


Fig.2 Means of feed intake (g/day/rat) in different groups of experiment (18th-22 week) in rats. Feed intake increased significantly in rats fed on HFD from (18th-22 week) compared with control group. Orlistat and herbal mix treatment tended to decrease feed intake compared with HFD group. Means have different letters indicate significant variation at ($P = .05$). Data expressed as mean $\pm$ SE with twelve animals in each group.

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 high fat diet caused a high significant increase ($P < .001$) 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 high fat diet and the other was fed on normal diet. We observed that the rats that were fed on high fat diet (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, table 3 and 4. But these increases in BW and BMI were changed by decreasing it with treatments of high fat diet with Orlistat and herbal mixture. When high fat diet treated with Orlistat and herbal mix from 18-22 weeks a significant ($P < .05$) and a high significant decrease ($P < .001$) occurred in these parameters respectively according to these figures. After the induction period of obesity finished (0-16 week), high fat diet began treated with Orlistat and herbal mix in 18-22 weeks. We observed that high fat diet had a high significant increase ($P < .001$) 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 high fat diet with Orlistat and herbal mix exerted a significant decrease in BW and BMI as shown in table 3 and 4.

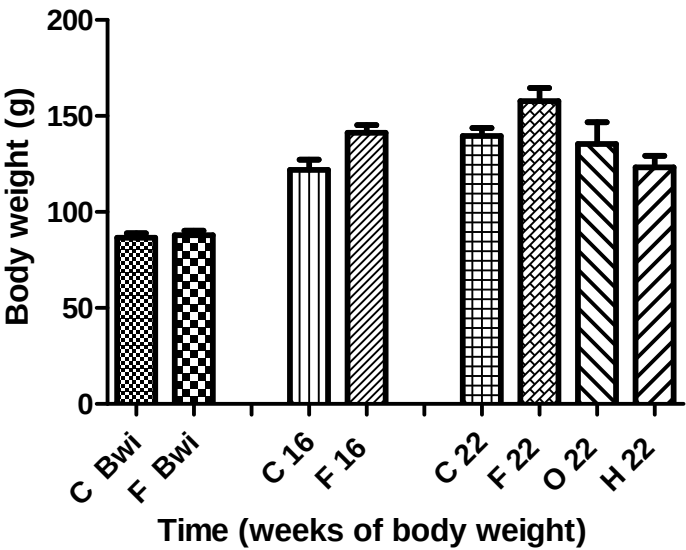
249 **Table 3. Effect of diet induced obesity and treatment on body weight:**

250

Groups	Weeks	Body weight (g)
Control	Initial Bwt	86.5 ± 2.4 ^a
HFD	Initial Bwt	87.8 ± 2.4 ^a
Control	16 th	121.9 ± 5.3 ^b
HFD	16 th	141.2 ± 4.0 ^b
Control	22	139.6 ± 4.1 ^b
HFD	22	157.8 ± 6.8 ^d
Orlistat	22	135.4 ± 11.3 ^b
Herbal mix	22	123.2 ± 6.1 ^b

251

252 Each value is the mean ± SEM. Means have different superscript letters indicate significant
253 variation at (P=.05), while the same letters indicate non-significant variation.



254

255 **Fig.3 Means of BW in rats during all period of experiment (22 weeks).** Mean BW
256 significantly increased during the period of the experiment (18th-22 weeks) in HFD compared
257 with that of control group. While Orlistat and herbal mix treatment significantly ameliorated
258 the changes during 18th-22 weeks compared with HFD group. Means have different letters
259 indicate significant variation at (P=.05). Data expressed as mean ± SE and number of animal
260 in each group is twelve.
261
262

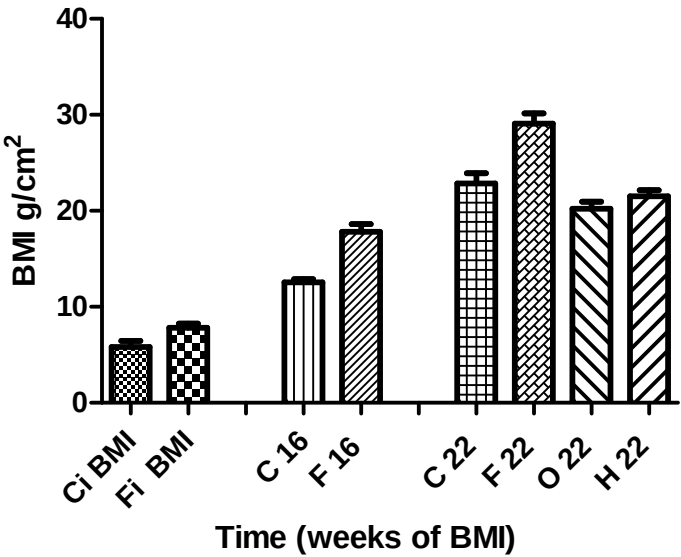
263 **Table 4. Effect of diet induced obesity and treatment on body mass index (BMI):**

264
265

Groups	Weeks	Body mass index (BMI) g/ cm ²
Control	Initial BMI	5.80 ± 0.62 ^a
HFD	Initial BMI	7.80 ± 0.44 ^a
Control	16 th	12.55 ± 0.34 ^b
HFD	16 th	17.81 ± 0.81 ^b
Control	22	22.85 ± 1.08 ^c
HFD	22	29.09 ± 1.04 ^d
Orlistat	22	20.20 ± 0.73 ^c
Herbal mix	22	21.50 ± 0.64 ^c

266 Each value is the mean ± SEM. Means have different superscript letters indicate significant
267 variation at (P=.05), while the same letters indicate non-significant variation.
268

269



270 **Fig.4 Means of BMI in rats during the period of the experiment (22 weeks).** BMI
271 significantly increased during the period of the experiment in HFD compared with that of
272 control group in rats. Administration of Orlistat and herbal mix significantly improved BMI
273 compared with HFD group. Means have different letters indicate significant variation at (P=
274 .05). Data expressed as mean ± SE and number of animal in each group is twelve.
275

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

Table 5 manifests the effect of HFD, Orlistat and herbal mix on serum cholesterol, triglyceride, LDL and HDL of high fat diet rats. High fat diet rats recorded a high significant increase ($P<.001$) in serum cholesterol, LDL. They also recorded a significant increase ($P<.01$) 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 high significant decrease ($P<.001$) in serum cholesterol and a significant decrease ($P<.05$) in serum triglyceride. Also, the treatment of HFD with herbal mix extract showed a significant decrease ($P<.05$) in serum cholesterol and triglyceride but two treatments for HDL and LDL showed a significant increase ($P<.05$) of serum HDL and a high significant decrease ($P<.001$) of serum LDL when compared to HFD as shown in table 5.

Table 5. 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

The number of animal in each group is twelve and data is expressed as mean ± SE. Identical letters indicates non-significance between groups, while different letters indicate significance between them at $P < .05$ when we compare high fat diet to control values and treating groups with high fat diet. Values between groups differ significantly: ⁺⁺⁺ $P<.001$, ⁺⁺ $P<.01$, ⁺ $P<.05$.

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

Table 6 manifests the effect of HFD, Orlistat and herbal mix on urea, creatinine and uric acid of high fat diet rats. The high fat diet rats exhibited no significant increase of urea and a significant increase ($P<.01$) 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 ($P<.05$) in creatinine. In addition, they recorded a non-significant decrease uric acid when compared to high fat diet rats as shown in table 6.

Table 6. 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	0.541 ± 0.036 ^a	1.964 ± 0.251 ^a
HFD	26.22 ± 1.55 ^a	0.682 ± 0.030 ^b	3.106 ± 0.161 ^b
Orlistat	23.19 ± 1.57 ^a	0.568 ± 0.017 ^a	2.864 ± 0.228 ^b
Herbal Mix	23.81 ± 1.25 ^a	0.560 ± 0.022 ^a	2.862 ± 0.148 ^b

The number of animals in each group is twelve and data is expressed as mean ± SE. Identical letters indicate non-significance between groups, while different letters indicate significance between them at $P < .05$ when we compare high fat diet to control values and treating groups with high fat diet. Values between groups differ significantly: ⁺⁺⁺ $P < .001$, ⁺⁺ $P < .01$, ⁺ $P < .05$.

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

Table 7 manifests the effect of HFD, Orlistat and herbal mix on renal oxidative stress of high fat diet rats. High fat diet rats exhibited a significant decrease ($P < .05$) of renal GSH and a high significant decrease ($P < .001$) of renal SOD. They also exhibited a high significant increase ($P < .001$) of renal MDA as compared to normal rats. While the treatment of HFD with Orlistat exerted a significant increase ($P < .05$) in renal GSH and SOD but a significant decrease ($P < .01$) in renal MDA as compared to high fat diet rats. In addition, the treatment of HFD with herbal mix exerted a high significant increase ($P < .001$) in renal GSH, a significant increase ($P < .05$) in renal SOD and a non-significant decrease in renal MDA when compared to HFD as shown in table 7.

Table 7. Effect of HFD, Orlistat and herbal treatment on renal oxidative stress:

Groups	Renal GSH (mg/dl)	Renal MDA (nmol/ml)	Renal SOD (u/gm)
Control	9.313 ± 0.211 ^a	35.48 ± 3.52 ^a	548.6 ± 24.1 ^a
HFD	7.736 ± 0.251 ^b	63.03 ± 1.48 ^b	375.6 ± 29.7 ^b
Orlistat	9.373 ± 0.225 ^a	50.33 ± 1.60 ^c	499.8 ± 27.6 ^a
Herbal Mix	10.420 ± 0.516 ^a	60.17 ± 0.02 ^b	462.0 ± 30.7 ^a

The number of animals in each group is twelve and data is expressed as mean ± SE. Identical letters indicates non-significance between groups, while different letters indicate significance between them at $P < .05$ when we compare HFD to control values and treating groups with HFD. Values between groups differ significantly: ⁺⁺⁺ $P < .001$, ⁺⁺ $P < .01$, ⁺ $P < .05$.

342

343 **Light Microscopic Examination of the Kidney:**

344 **Group (1) the kidney of control rats:**

345 A histological examination of the normal kidney showed that it consists of an outer cortex
346 an inner medulla. The kidney cortex revealed the typical histological structure of the renal
347 corpuscle and the renal tubules. The renal corpuscle includes the glomerulus which consists
348 of small tuft of fenestrated capillaries covered by thin diaphragm and Bowman's capsule fig.
349 5 a&b. The capsule consists of an inner visceral layer and an outer parietal layer. Also, the
350 renal tubules consist of proximal convoluted tubules which are lined with a brush border
351 cuboidal epithelium. While the distal convoluted tubules are lined with low and non-brush
352 border cuboidal epithelial cells.

353 **Group (2) rats treated with high fat diet:**

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

359 **Group (3) rats treated with high fat diet with Orlistat:**

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

368 **Group (4) rats treated with high fat diet with pumpkin oil and nutshell extract:**

369 Animals treated with high fat diet plus pumpkin oil and nutshell extract showed a
370 histological picture more or less similar to controlled group fig. 6 k. While others showed
371 some hemorrhage between renal tubules fig. 6l.

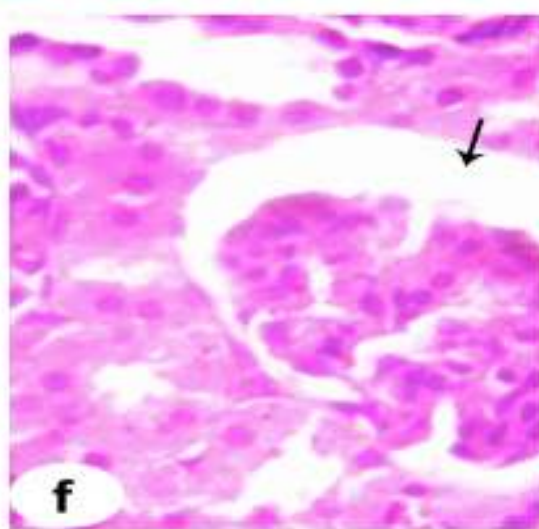
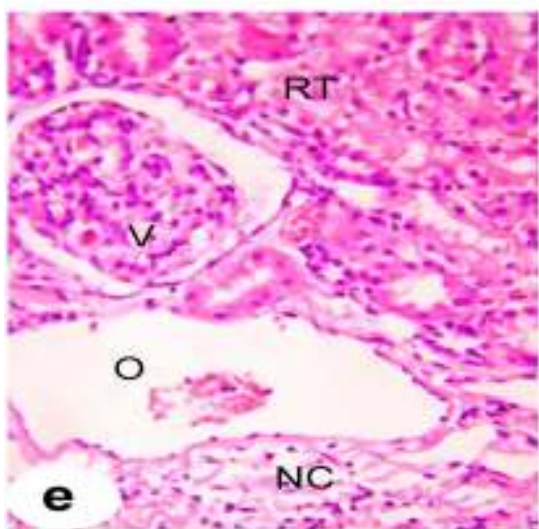
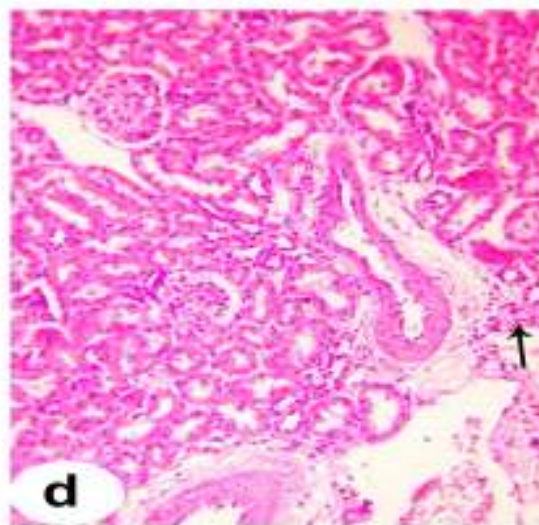
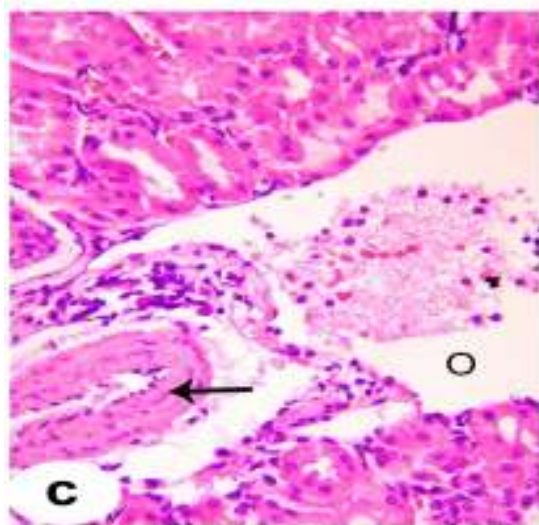
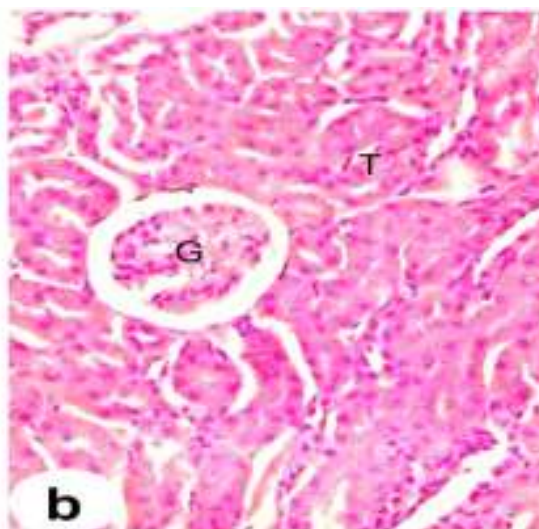
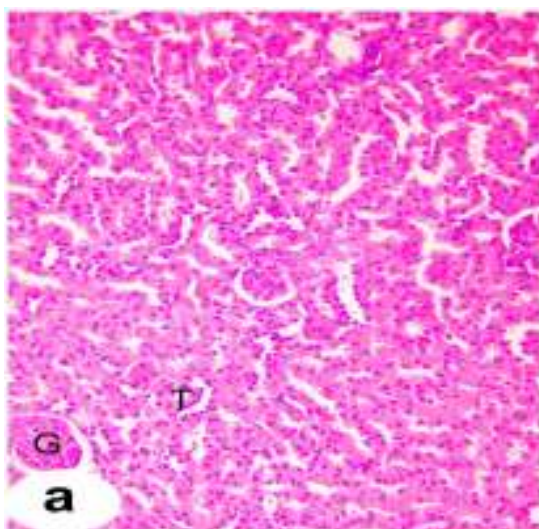
372 **Figure 5: Photomicrograph of the kidneys sections of normal rats (a, b) and**
373 **treated with high fat diet (c, d, e and f):**

374

375

376

377



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

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

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

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

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

392

393 **Figure 6: Photomicrograph of the kidneys sections of rats treated with high fat**
394 **diet plus Orlistat (g, H, I, j) and herbal extract (k, l):**

395

396

397

398

399

400

401

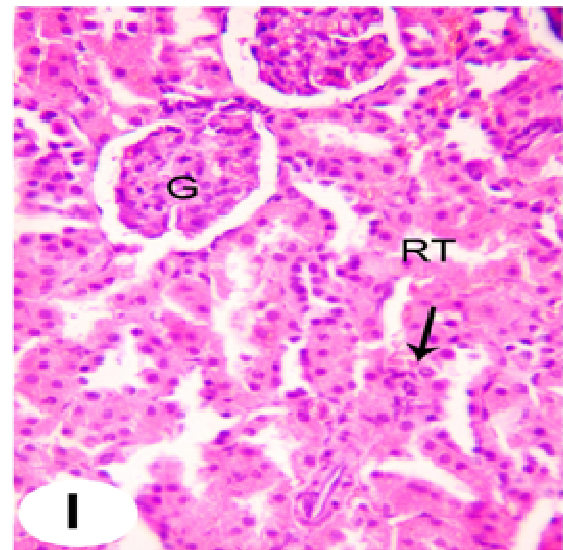
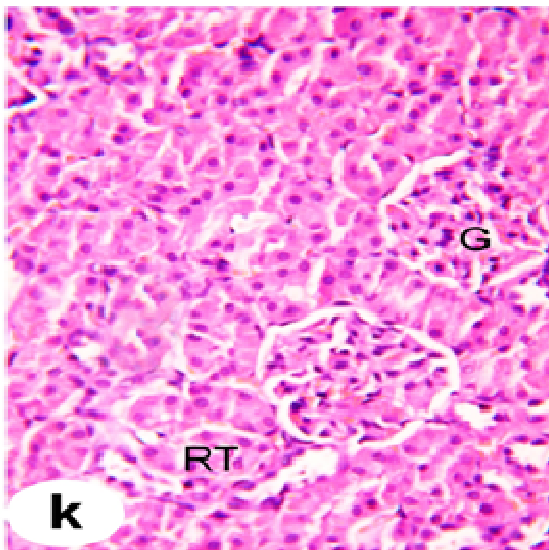
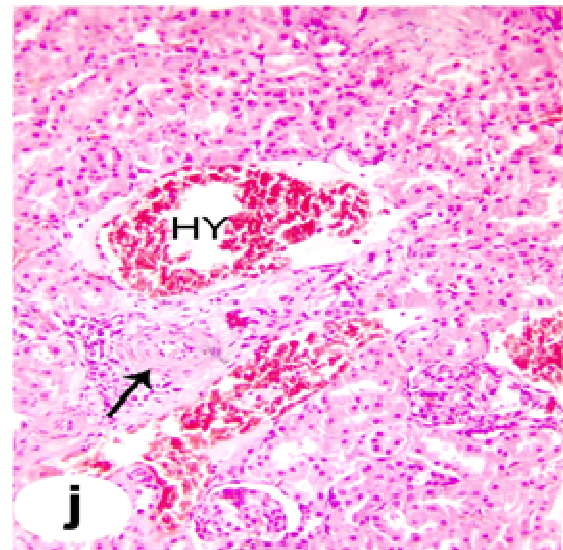
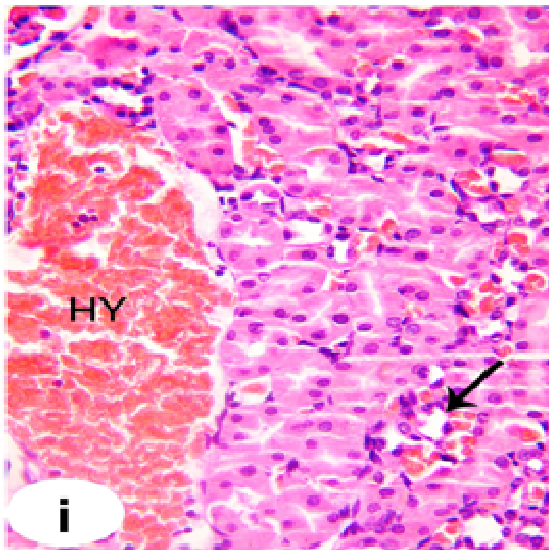
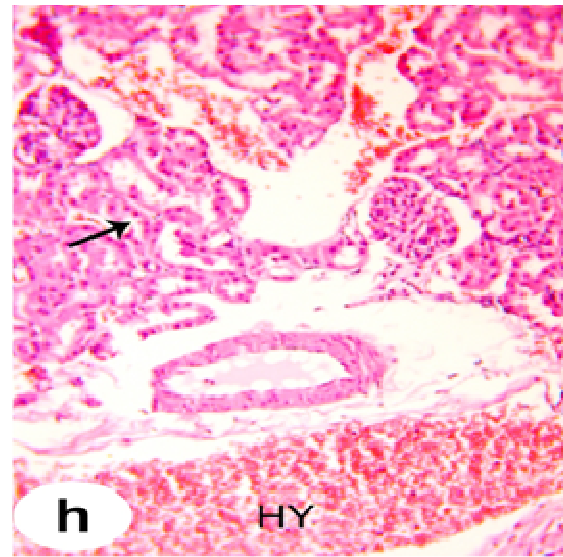
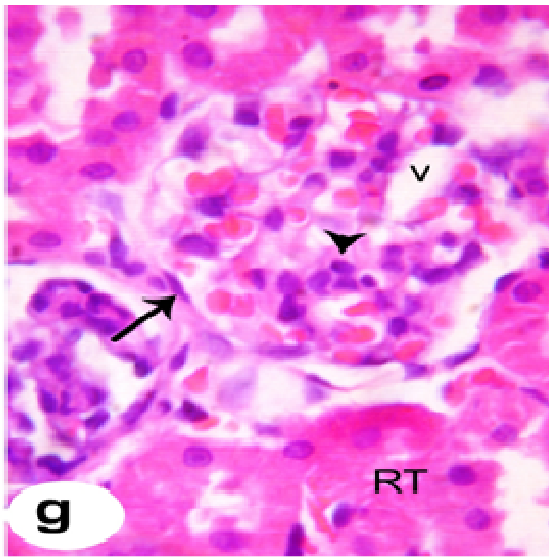


Fig (6 g): Photomicrograph of the kidneys' sections of rats treated with high fat diet plus Orlistat showing hyperemic glomerular tuft (▲), swelling in lining epithelium of renal tubules (RT) and vacuolar glomerular tuft (V) with swollen endothelial cells lining the Bowman's capsule (↑). H & E x 400.

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

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

440

441 **3.2 DISCUSSION**

442

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 energy 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 occurred 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 pumpkin oil 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 peanut shells (PSE) extracts 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 high fat diet to male Wistar rats with 1% of PSE [11].

Our study indicated that **obesity** leads to morbidity as well as mortality. There is usually increased level of total cholesterol, LDL-cholesterol, triglycerides and decreased level of HDL-cholesterol. 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). Low density lipoprotein-high density lipoprotein (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 rats with pumpkin oil 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 high fat diet 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 pumpkin oil provided lower total cholesterol, lower (LDL) cholesterol, lower triglycerides and higher (HDL) cholesterol levels. **Pumpkin oil may reduce (LDL) (bad) cholesterol. The oil may also lower systolic and diastolic blood pressures due to its high concentration of a phytoestrogens [33].** Pumpkin oil (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 high fat diet 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.**

High fat diet (HFD) caused a significant elevation in serum creatinine table 6; 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

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

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

546 On the other hand treatment with extract of peanut shell caused reduction in values of urea
547 nitrogen and creatinine due to the active component luteolin that found in it [40]. There was a
548 concomitant significant reduction in the elevated of marker parameters of kidney toxicity
549 BUN and serum creatinine in luteolin pretreatment groups. This showed the efficacy of
550 luteolin in regulating renal functions [40]. Luteolin increases the level of HO-1 protein. It was
551 suggested that one of the mechanism of the renoprotective effect of luteolin may be related
552 to increasing HO-1 expression and elevating antioxidant in diabetic nephropathy. Peanut
553 shell extract significantly reduced renal oxidative damage [7] and had a strong renal
554 protective effect against glycerol-induced acute renal failure (ARF).

555 Dietary HFD caused a significant decrease of kidney GSH, kidney SOD and a high
556 significant increase of kidney MDA as compared to the normal rats. Oxidative stress is
557 caused by an imbalance between increased production of reactive oxygen species (ROS)
558 and/or reduced antioxidant activity, leading to oxidative damage to cells or tissue including
559 lipids, proteins and DNA. Studies had suggested that obesity is associated with increased
560 oxidative stress. Oxidative stress had been commonly identified in obesity-related renal
561 diseases and may be the mechanism underlying the initiation or progression of renal injury in
562 obesity [41]. ROS are highly reactive molecules that oxidize lipids and proteins, cause
563 cellular injury and promote glomerular and renal tubule injury and associated proteinuria.
564 High fat diet induced obesity is accompanied by increased renal tissues oxidative stress,
565 which is characterized by reduction in the antioxidant enzymes activities and glutathione
566 levels, that correlate with the increase in MDA and protein carbonyl (PCO) levels in most
567 tissues [42].

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

576 On the other hand treatment of HFD rats with herbal mix produced a significant increase in
577 kidney GSH, SOD and a high significant decrease in kidney MDA as compared to the high
578 fat diet rats. Wang et al. [7] reported that pretreatment with peanut shell extract (EPS) in
579 acute renal failure (ARF) rats caused GSH in serum and renal cortical significantly
580 increased. In addition, renal cortex homogenate MDA levels were significantly lower also,
581 the treatment with pumpkin oil contained omega-3 caused significantly lower for oxidative
582 stress which had increased following reperfusion injury [43]. Preventive oral administration of
583 omega-3 supplement may decrease oxidative stress, kidney dysfunction following
584 reperfusion injury and may result in better anti-oxidation status.

585 **Photomicrograph of the kidneys sections of normal HFD, Orlistat and herbal** 586 **treatment:**

587 Obesity is a chronic metabolic disorder that results from the imbalance between energy
588 intake and energy expenditure characterized by enlarged fat mass and elevated lipid
589 concentration in blood. Many attempts had been made to correct the metabolic disparity of
590 the obesity condition, producing a number of reagents including fibrates, sibutramine (an
591 anorectic or appetite suppressant) and Orlistat but no drug is free from severe side effects
592 [44]. The present study demonstrates that a high-fat diet, fed for as little as 0 to 22 wk, figure
593 5 produced significant histologic, biochemical and functional changes in the kidney in
594 comparison with normal group. These changes included expansion of Bowman's capsule.
595 The mechanisms responsible for the enlargement of Bowman's space in obesity are
596 uncertain but may be related to the marked glomerular hyperfiltration; it is interesting that the
597 GFR was increased by almost the same percentage (38%) as the Bowman's space
598 increase. In our histological study, dilatation of glomerular capillaries and large blood vessels
599 had been seen. In addition to tubular deformations, histologically detected glomerular
600 atrophy, necrosis and oedema similar results were reported by Altunkaynak et al. [45]. The
601 increase in the volume of the kidneys of HFD-fed rats may have resulted from oedema due
602 to mononuclear cell infiltrations among the tubules. It is clearly understandable that dilatation
603 may lead to a volumetric increase in the kidney. According to Hvizdos and Markham [46],
604 Orlistat is a novel non-systemic treatment for obesity it inhibits lipases in the gastrointestinal
605 tract, preventing the absorption of approximately 30% of dietary fat. The present study
606 revealed that the administration Orlistat revealed that focal necrotic area with inflammatory
607 cells infiltration and blood vessels show congestion and some renal tubules suffer from
608 degeneration. Similar results were reported by Korzets et al. [47] who found that renal biopsy
609 findings showed that the chronic kidney disease (CKD) was attributable to
610 nephrocalcinosis/oxalate nephropathy, resulting in a chronic tubulo-interstitial nephropathy,
611 while acute tubular necrosis. HFD increase serum uric acid table 6 that initiate renal urate
612 crystal producing histopathological changes.

613 Orlistat was capable of increasing urinary oxalate levels many-fold, especially in rats fed
614 high fat or oxalate-enriched diet. A rise in the ion-activity product of calcium oxalate was also
615 demonstrated. Interestingly, no renal depositions of oxalate were seen on examination of
616 renal tissue after termination 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 (FPSO), 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 via elevating 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.

ACKNOWLEDGEMENTS

This study was supported by Beni Suef University. We appreciate the support offered by department of biochemistry

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' managed the analyses of the study and parts of histopathology. 'Author 4' managed the literature searches..... All authors read and approved the final manuscript."

CONSENT (WHERE EVER APPLICABLE)

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

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

669 specific national laws where applicable. All experiments have been examined and approved
670 by the appropriate ethics committee"

671

672

673 For manuscripts involving human experiments, "All authors hereby declare that all
674 experiments have been examined and approved by the appropriate ethics committee and
675 have therefore been performed in accordance with the ethical standards laid down in the
676 1964 Declaration of Helsinki."

677

678 **REFERENCES**

679

[1]. World Health Organization. Obesity and overweight. Fact sheet N°311. September
2006. mediainquiries@who.int. 680 681

682 [2]. Gonzalez E, Gutierrez E, Morales E, Hernandez E, Andres A, Bello I, Diaz-Gonzalez R,
683 Leiva O, Praga M. Factors influencing the progression of renal damage in patients with
684 unilateral renal agenesis and remnant kidney. Kidney International. 2005; 68(1):263-270.
685 [DOI:10.1111/j.1523-1755.2005.00401.x](https://doi.org/10.1111/j.1523-1755.2005.00401.x). 686

687 [3]. Quigley JE, Elmarakby AA, Knight SF, Manhiani MM, Stepp DW, Olearczyk JJ, Imig JD.
688 "Obesity induced renal oxidative stress contributes to renal injury in salt-sensitive
689 hypertension." Clinical and Experimental Pharmacology and Physiology. 2009; 36(7):724–
690 728. [DOI:10.1111/j.1440-1681.2009.05139.x](https://doi.org/10.1111/j.1440-1681.2009.05139.x). [PMCID]: [PMC2710419](https://pubmed.ncbi.nlm.nih.gov/PMC2710419/).

[4]. Asghar M, Monjok E, Kouamou G, Ohia SE, Bagchi D, Lokhandwala MF. Superoxide
max (HCA-SX) attenuate increase in oxidative stress, inflammation, insulin resistance and body
weight in developing obese Zucker rats. Molecular and Cellular Biochemistry. 2007; 298(1-
2):93-99. [DOI: 10.1007/s11010-007-9489-3](https://doi.org/10.1007/s11010-007-9489-3). 691 692 693 694 695

696 [5]. Eagles JM. Treatment of depression with pumpkin seeds. British Journal of Psychiatry.
697 1990; 157:937-8. [PMID]: [2289114](https://pubmed.ncbi.nlm.nih.gov/2289114/). 698

699 [6]. Brasaemle DL, Barber T, Wolins NE, Serrero G, Blanchette-Mackie EJ, Londos C.
700 Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-
701 associated protein. Journal of Lipid Research. 1997; 38(11):2249–63. [PMID]: [9392423](https://pubmed.ncbi.nlm.nih.gov/9392423/).

702 [7]. Wang M, Zhou X, Chu Y. Effects of peanut shell extract on rats with acute renal failure.
703 Remote Sensing, Environment and Transportation Engineering (RSETE). International
704 Conference on Digital Object Identifier. 2011; 8264–8267. DOI:
705 [10.1109/RSETE.2011.5964079](https://doi.org/10.1109/RSETE.2011.5964079).

706 [8]. Kim MS, Kim Jk, Kwon DY, Park R. Antiadipogenic effect of garcinia extract on lipid
707 droplets accumulation and the expression of transcription factor. Bio factor. 2004; 22:193-
708 196. PMID: [15630282](https://pubmed.ncbi.nlm.nih.gov/15630282/) [PubMed].

709 [9].The internet drug index RxList. Xenical (Orlistat 120 mg) Drug Information: Description,
710 User Reviews, Drug Side Effects and Drug Interactions. 2012. Available:
711 www.rxlist.com/xenical-drug.htm. 712

- [10]. Kumar S, Alagawadi KR. Effect of *Argyrea speciosa* roots on atherogenic diet induced obesity in rats. *Journal of Pharmacy Research*. 2010; 3(7):1524-1527. 714
- 715 [11]. Moreno DA, Ilic N, Poulev A, Raskin I. Effects of *Arachis hypogaea* nutshell extract on
716 lipid metabolic enzymes and obesity parameters. *Life Sciences*. 2006; 78(24):2797-803.
717 PMID: 16337240 [PubMed].
- 718 [12]. Zuhair HA, Abd El-Fattah AA, El-Sayed MI. Pumpkin-seed oil modulates the effect of
719 felodipine and captopril in spontaneously hypertensive rats. *Pharmacological Research*.
720 2000; 41(5):555-63. PMID: 10753555 [PubMed].
721
- 722 [13]. Roberts CK, Berger JJ, Barnard RJ. Long-term effects of diet on leptin, energy intake
723 and activity in a model of diet-induced obesity. *Journal of Applied Physiology*. 2002; 93:887-
724 893. PMID: 12183482 [PubMed].
725
- [14]. Drury RAB, Willington EA. *Carleton's histological techniques*. 8* ed. Oxford University;
1980. 727
- 728 [15]. Schettler G, Nussel E. Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing
729 Factor Determination of total cholesterol. *Arb. Med. Soz. Med. Prav. Med.* 1975; 10:25.
- 730 [16]. Gordon T, Gordon M. Enzymatic method to determine the serum HDL cholesterol by
731 Precipitant and Standard, For Use with CHOLESTEROL Liquicolor Test Kit. *American*
732 *Journal of Medicine*. 1977; 62: 707-708.
733
- 734 [17]. Fassati P, Prencipe L. Enzymatic Colorimetric Test for Triglycerides. *Clinical Chemistry*.
735 1982; 28:2077.
736
- 737 [18]. Young DS. Effect of drugs on clinical lab. tests, 4th ed. Berthelot Enzymatic Colorimetric
738 Method Determination of Urea aacc press; 1995.
- 739 [19]. Labbe D, Vassault A. Kinetic determination of creatinine without deproteinization in
740 human urine, serum and plasma. 2000. Available: [http://bioch.ap-hop-](http://bioch.ap-hop-paris.fr/analyses/bioforma/creatinine.htm)
741 [paris.fr/analyses/bioforma/creatinine.htm](http://bioch.ap-hop-paris.fr/analyses/bioforma/creatinine.htm).
742
- 743 [20]. Barham D, Trinder P. Enzymatic colorimetric for Determination of uric acid. *Analyst*.
744 1972; 97:142.
745
- 746 [21]. Beutler E, Duron O, Kelly MB. Colorimetric method for Determination of Glutathione
747 reductase concentration. *Journal of Laboratory and Clinical Medicine*. 1963; 61:882.
- 748 [22]. Nishikimi M, Roa NA, Yogi K. Colorimetric method for Determination of superoxide
749 dismutase activity (SOD). *Biochemical and Biophysical Research Communications*. 1972;
750 46:849-854.
- 751 [23]. Ohkawa H, Ohishi W, Yagi K. Colorimetric method for Determination of MDA activity.
752 *Biochemistry*. 1979; 95:351.
753
- 754 [24]. Haslam DW, James WP. Obesity. *Lancet*. 2005; 366(9492):1197-1209. PMID:
755 16198769 [PubMed].

- 756 [25]. Weisinger JR, Kempson RL, Eldridge FL, Swenson RS. "The nephrotic syndrome: a
757 complication of massive obesity." *Annals of Internal Medicine*. 1974; 81(4):440–447, doi:
758 [10.7326/0003-4819-81-4-440](https://doi.org/10.7326/0003-4819-81-4-440).
- 759 [26]. Chagnac A, Weinstein T, Korzets A, Ramadan E, Hirsch J, Gafer U. "Glomerular
760 hemodynamics in severe obesity." *American Journal of Renal Physiology*. 2000;
761 278(5):F817-22. PMID: 10807594 [PubMed].
- 762 [27]. La fleur SE, Van Rozen AJ, Groen Weg E, Adan RA. A free choice high fat, high sugar
763 diet induces changes in arcuate neuropeptides expression that support hyperphagia.
764 *International Journal of Obesity*. 2009; 22(4):55-63, doi: [10.1038/ijo.2009.257](https://doi.org/10.1038/ijo.2009.257), PMID:
765 [20029382](https://pubmed.ncbi.nlm.nih.gov/20029382/) [PubMed].
- 766 [28]. Akiyama T, Tachibana I, Shirohara H, Watanabe N, Otsuki M. High-fat hypercaloric diet
767 induces obesity, glucose intolerance and hyperlipidemia in normal adult male Wistar rat.
768 *Diabetes Research and Clinical Practice*. 1996; 31(1– 3):27–35. PMID: 8792099 [PubMed].
- 769 [29]. Drent ML, van der Veen EA. First clinical studies with Orlistat: a short review. *Obesity*
770 *Research*. 1995; 3 Suppl 4: 623S-625S. PMID: 8697067 [PubMed].
- 771 [30]. Supplementation with PSO. *Phytotherapy Research*. 2008; 22(7):873-7.
- 772 [31]. Szczypińska A, Widomska S, Jaraszkiewicz M, Knera P, Muc K. Blood lipids profile in
773 obese or overweight patients. *Annales Universitatis Mariae Curie Skłodowska Sectio D*
774 *Medicina*. 2003; 58(2):343-9. PMID: 15323217 [PubMed].
- 775 [32]. Drent ML, Larsson I, William-Olsson T, Quaade F, Czubyko F, von Bergmann K,
776 Strobel W, Sjöström L, van der Veen EA. Orlistat (RO 18-0647), a lipase inhibitor, in the
777 treatment of human obesity: a multiple dose study. *International journal of obesity and*
778 *related metabolic disorders*. 1995; 19(4):221-6. PMID: 7627244 [PubMed].
- 779 [33]. Gossell-Williams M, Lyttle K, Clarke T, Gardner M, Simon O. Supplementation with
780 Pumpkin Seed Oil Improves Plasma Lipid Profile and Cardiovascular Outcomes of Female
781 Non-Ovariectomized and Ovariectomized Sprague-Dawley Rats." *Phytotherapy Research*.
782 2008; 22(7):873-7, doi: [10.1002/ptr.2381](https://doi.org/10.1002/ptr.2381), PMID: 18567058 [PubMed].
- 783 [34]. Moreno DA, Ilic N, Poulev A, Brasaemle DL, Fried SK, Raskin I. Inhibitory effects of
784 grape seed extract on lipases. *Nutrition*. 2003; 19(10):876–879. PMID: 14559324 [PubMed].
- 785 [35]. Matsushita K, Yasuda G, Shouda M, Umemura S. Evaluation of renal function and
786 protein urea body mass health examination in young obese Japanese people. *Clinical and*
787 *Experimental Nephrology*. 2009; 13(4):316-324, doi: [10.1007/s10157-009-0164-8](https://doi.org/10.1007/s10157-009-0164-8), PMID:
788 [19377907](https://pubmed.ncbi.nlm.nih.gov/19377907/) [PubMed].
- 789 [36]. Prior LJ, Eikelis N, Armitage JA, Davern PJ, Burke SL, Montani JP, Barzel B, Head GA.
790 Exposure to a high-fat diet alters leptin sensitivity and elevates renal sympathetic nerve
791 activity and arterial pressure in rabbits. *Hypertension*. 2010; 55(4):862-868, doi:
792 [10.1161/HYPERTENSIONAHA.109.141119](https://doi.org/10.1161/HYPERTENSIONAHA.109.141119). PMID: 20194306 [PubMed].
- 793 [37]. Must A. Morbidity and mortality associated with elevated body weight in children and
794 adolescents. *The American Journal of Clinical Nutrition*. 1996; 63(3):445S-447S. PMID:
795 [8615339](https://pubmed.ncbi.nlm.nih.gov/8615339/) [PubMed].
- 796

- 797 [38]. Shaheen KA. Weight loss of experimental animals by brown algae in comparison with a
798 commercial medical preparation. Faculty of Home Economics, Menoufiya University, Egypt.
799 2007; pp. 18–36.
- 800 [39]. Barakat LA, Mahmoud RH. The antiatherogenic, renal protective and
801 immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic
802 rats. North American Journal of Medical Sciences. 2011; 3(9):411–417, doi:
803 [10.4297/najms.2011.3351](https://doi.org/10.4297/najms.2011.3351). PMID: 22362450 [PubMed] PMCID: PMC3271396.
- 804 [40]. Sultana S, Prasad L, Jahangir T. Lutoelin ameliorates ferric nitrilotriacetic acid induced
805 renal toxicity and tumor promotional response in rats. Indian Journal of Experimental
806 Biology. 2009; 47(5):355-360. PMID: 19579801 [PubMed].
- 807 [41]. Darouich S, Goucha R, Jaafoura MH, Zekri S, Ben Maiz H, Kheder A.
808 “Clinicopathological characteristics of obesity associated focal segmental
809 glomerulosclerosis.” Ultrastructural Pathology. 2011; 35(4):176–182, doi:
810 [10.3109/01913123.2011.584657](https://doi.org/10.3109/01913123.2011.584657). PMID: 21657818 [PubMed].
- 811 [42]. Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress markers
812 in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetology & Metabolic
813 Syndrome. 2011; 3(1):17, doi: [10.1186/1758-5996-3-17](https://doi.org/10.1186/1758-5996-3-17). PMID: 21812977 [PubMed] PMCID:
814 [PMC3174870](https://doi.org/10.1186/1758-5996-3-17).
- 815 [43]. Ashtiyani SC, Najafi H, Kabirinia K, Vahedi E, Jamebozorky L. Oral omega-3 fatty acid
816 for reduction of kidney dysfunction induced by reperfusion injury in rats. Iranian Journal of
817 Kidney Diseases. 2012; 6(4):275-83. PMID: 22797097 [PubMed].
- 818 [44]. Tziomalos K, Krassas GE, Tzotzas T. The use of sibutramine in the management of
819 obesity and related disorders: an update. Journal of Vascular Health and Risk Management.
820 2009; 5(1):441–452. PMID: 19475780 [PubMed] PMCID: PMC2686261.
- 821 [45]. Altunkaynak ME, Özbek E, Altunkaynak BZ, Can İ, Unal D, Unal B. The effects of high-
822 fat diet on the renal structure and morphometric parametric of kidneys in rats. Journal of
823 Anatomy. 2008; 212(6):845–852, doi: [10.1111/j.1469-7580.2008.00902.x](https://doi.org/10.1111/j.1469-7580.2008.00902.x), PMCID:
824 [PMC2423405](https://doi.org/10.1111/j.1469-7580.2008.00902.x).
- 825
826 [46]. Hvizdos KM, Markham A. Orlistat: a review of its use in the management of obesity.
827 Drugs. 1999; 58(4):743-60. PMID: 10551441 [PubMed].
- 828 [47]. Korzets A, Gafter U, Tobar A, Chagnac A, Zingerman B, Ori Y. Furosemide, Orlistat
829 and non-steroidal anti-inflammatory agents—too much for the kidneys to handle!. NDT Plus.
830 2009; 2(2):167–170, doi: [10.1093/ndtplus/sfn217](https://doi.org/10.1093/ndtplus/sfn217).
- 831 [48]. Karamadoukis L, Ludeman L, Williams AJ. Is there a link between calcium oxalate
832 crystalluria, Orlistat and acute tubular necrosis? Nephrology Dialysis Transplantation. 2008;
833 23(5):1778–1779, doi: [10.1093/ndt/gfm945](https://doi.org/10.1093/ndt/gfm945). PMID: 18272781 [PubMed].
- 834
835

836 DEFINITIONS, ACRONYMS, ABBREVIATIONS

837 **Term:** Definition for the term

838

839 APPENDIX

840

Term	Definition for the term
AngII	Angiotensin II
ARF	Acute renal failure
BMI	Body mass index
BUN	Blood urea nitrogen
BW	Body weight
CH	Cholesterol
CKD	Chronic kidney disease
Cre	Creatinine
EPS	Extract peanut shell
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	Malonaldehyde
Nc	Necrotic cells

ORG	Obesity-related glomerulopathy
PAF	Platelet-activating factor
PL	Pancreatic lipase
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

841
842
843
844
845
846
847