

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: Obesity became major health problem in the world it is risk factor for renal disease. **Aim of work:** the objective of this work was to examine the effect of high fat diet to induce obesity on antioxidant defense system, biochemical changes in blood and tissue of control, non-treated and treated groups by administration of Orlistat and herbal mixture (pumpkin oil+nutshell extract) and explore the mechanisms that link obesity with altered renal function.

Materials and Methods: Rats were fed a standard control diet for 22 week (1st control negative group) or a diet containing 35% fat (HFD) for 16 week and then HFD group divided into 3 groups for the following 6 wks. One group was HFD, the 3rd group HFD+Orlistat and 4rd HFD + herbal mix (pumpkin oil and nutshell extract). Blood and renal, mesenteric, Perirenal and epididymal adipose tissues were collected for biochemical assays.

Results: HFD group of rats showed a significant increase in feed intake, body weight (BW) and body mass index (BMI). Also there were significant increases in weights of mesenteric, Perirenal and epididymal adipose tissues in HFD in comparison with normal control group. HFD affect the kidney by increasing serum urea, uric acid, creatinine levels and increased blood glucose, low density lipoproteins (LDL), triacylglycerol (TG), total cholesterol (TC) and malondialdehyde (MDA). These changes improved by Orlistat and herbal mixture that decreased the oxidative stress biomarkers. There were significant positive correlations among BMI, kidney functions (Creatinine and urea), TG and oxidative markers (renal MDA).

Conclusions: Rats fed HFD showed, hypertriglyceridemia, increased LDL production, 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, MDA level and decreased oxidative stress in renal tissues.

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

Introduction

Obesity has been one of the leading public health concerns in industrialized societies for the last 40 years. There is an increasing prevalence in the USA as well as in Europe and this is steadily progressing to the rest of the world. Obesity among adults has been defined by the WHO as a body mass index or BMI above 30 kg/m² (1). Children and adolescents regarded as overweight

are recorded as $\geq$ 95th percentile of the sex-specific BMI for their age growth charts **(2)**.

The association between obesity and the development of renal diseases is documented. In a large Japanese cohort ($n > 100000$), followed up after 17 years, reported that obesity increased the risk of end-stage renal disease (ESRD) among men. This relationship persisted even after adjustments for hypertension and proteinuria, suggesting that obesity *per se* is an independent risk factor for ESRD **(3)**.

A large cross-sectional population study demonstrated that being overweight, from the age of 20 to any time later in life, was linked with an increased risk of chronic kidney disease (CKD) for both men and women. In addition, in this cohort, the risk for CKD in obese people considerably increased the risks of hypertension and type 2 diabetes **(4)**. Excess weight plays a fundamental role in the development of proteinuria and renal damage in patients with severe renal mass reduction **(5)**. Furthermore, it was demonstrated that obesity accelerates the increase of proteinuria in IgA nephropathy through remodelling of the glomerular membrane **(6)**. Therefore, these previous publications demonstrate mediate statistical and renal physiological analysis of the role of obesity as a precursor or contributor to renal diseases. Several clinical studies have also found that the risk for 35 micro albuminuria was independent of hypertension and associated with different components of metabolic syndrome, such as abdominal obesity and BMI **(7&8)**.

The combination of insulin resistance, hyperinsulinemia and abdominal obesity before the development of clinical diabetes might be a critical risk factor in the development of CKD **(9)**. While the mechanism of obesity on renal changes need more investigation concerning the role of oxidative stress

in developing renal changes and comparative possible treatments between drug and natural herbal extract.

This study investigates uncharacterized 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 (pumpkin oil + nutshell extract) on it.

Materials and Methods

Diet:

Two types of diets have been used, control rat chow diet and special high fat diet HFD (35 %) for induction of obesity in rats.

Chemicals:

Hard capsule: turquoise cap and turquoise body bearing the imprint (Roche Xenical 120) was given orally by oral gavage with a dose 2 mg /kg bw/day.

Herbal mixture:

1- Pumpkin oil: was purchased from a local market, it is a liquid is given by oral gavage with dose 5mg/kg bw/day.

2- Ethanolic extract of Nutshell was purchased from a local market then the extract was used 2 mg/kg bw/day.

Methods

8-3-1 Animals grouping:

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

This study was carried out on white male albino rats (wistar rats) weighing about 100 ± 20 gm were used as experimental animals. Rats were fed a standard control diet for 22 weeks. Fifty male rats were divided into four groups:

1st a normal diet group (control negative).

2nd High fat diet group (HFD) act as control positive.

3rd HFD with 2 mg/kg bw /day Orlistat (for 6 weeks).

4th HFD with 5 mg/kg bw/day pumpkins and 2 mg/kg bw/day nutshell extract (for 6 weeks).

Sampling and tissue preparation:

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 and perirenal, mesenteric and epididymal adipose tissues. Kidney was taken and washed by saline (0.9%), dried by filter paper and weighed 0.5 gm 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 xylene and then embedded in paraffin wax. Sections (5µm thick) were stained with haematoxylin and eosin (10).

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) (11).

HDL cholesterol in serum determined according to the method of Precipitant and Standard, for Use with Cholesterol Liquicolor Test Kit **(12)**.

Cholesterol and HDL determine by using the reagent kits purchased from Human Gesellschaft fur Biochemical and Diagnostic mbH. Max-planch – ring 21-65205 Wiesbaden. Germany.

Serum TG was determined according to the method of **(13)** and GPO-PAP-Method Enzymatic Colorimetric Test for Triglycerides with Lipid Clearing Factor (LCF). Using kits from Bio Diagnostic company 29 Tahreer St, Dokki, Giza, Egypt. And Human Gesellschaft fur Biochemical and Diagnostic mbH.

LDL cholesterol was determined according to the equation: LDL Cholesterol (mg%)=total Cholesterol–(triglyceride/5)-HDL Cholesterol.

The concentration of urea was determined according to Berthelot. Enzymatic Colorimetric by using the reagent kits from 23 El-Montzh St. Heliopolis, Cairo, Egypt **(14)**.

Determination of Creatinine by using the reagent kits from 23 El-Montzh St, Heliopolis, Cairo, Egypt **(15)**.

Uric acid level was determined according to Enzymatic Colorimetric Method by using the reagent kits from Bio-diagnostic Company 29 Tahreer St, Dokki, Giza, Egypt **(16)**.

GSH level was measured, as described by **(17)**.

SOD activity was determined according to Colorimetric Method using the reagent kits from Bio-diagnostic Company 29 Tahreer St, Dokki, Giza, Egypt. SOD activity was assayed as described by **(18)**.

MDA concentration was determined according to Colorimetric Method by using the reagent kits from Bio-diagnostic company 29 Tahreer St, Dokki, Giza, Egypt (19).

Statistical analysis of the results:

Data were presented as mean $\pm$ S.E.M and analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer methods for post-hoc analysis. A value of $p < 0.05$ was considered statistically significant. Graph Pad Prism 5 software (San Diego, CA, USA) was used for statistical analysis.

Results

1- Effect of HFD, Zenical and herbal mix. on feed intake:

In this study, feed intake were significantly increased during the period of the experiment in accompanied with significant increases in mesenteric, perirenal and epididymal adipose tissue in HFD compared with that of control group in rats. Zenical and herbal mix treatment tended to decrease food intake (Table 1 and 2).

Data of HFD group during 18 weeks showed a significant increase in feed intake compared to control group (Figure 1), while treatment with Orlistat and herbal mix exerted non significant decrease in feed intake and weight loss. While with continuous feeding of rats on high fat diet and treatment of its with Orlistat and herbal mix occurred a continuous increasing in feed intake with these rats that feed on high fat diet than control group. Also, a significant decrease in feed intake with these rats that treated with Orlistat and herbal mix and this occurred in (20 week). At the end of experimental study (22 week) we observed that HFD group continuously increased

significantly in feed intake compared to control group, while treatment Orlistat and herbal mixture showed a significant decrease in feed intake (Table 1 and 2).

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

Our data showing there was significant higher body weight in free diet of HFD during compared to control diet. Also, high fat diet caused a significant increase in body weight and body mass index from 0-16th to 18-22 wk. This means that the high fat diet increased body weight and body mass index in all experimental period (from initial 0 to 22 week).

In the beginning of the experiments (initial weight) all weight of two groups was the same (control and HFD) and showed non significant between them in weight. But these weights changed fastly when we feed one of them on high fat diet and another feed on normal diet. We observed that the rats that feed on high fat diet (HFD) group 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 week) (Table 3 and 4).

But these increases in body weight and body mass index were changed by decreasing both body weight and body mass index with treatments of high fat diet with Orlistat and herbal mixture. When high fat diet treated with Orlistat and herbal mix from 18-22 week occurred a significant decrease in these parameters according to these figures. After induction period of obesity finished (0-16 week) high fat diet began treated with Orlistat and herbal mix (18-22 week). We observed that high fat diet continuously increase in body weight and body mass index to the final of experiments (16-22 week) compared to control group. But when treatment started (18-22) our results

showed that the treatment of high fat diet with Orlistat and herbal mix exerted a significant decrease in body weight and body mass index (Table 3 and 4).

3- Effect of HFD, Zenical and herbal treatment on serum cholesterol and TG in rats:

Data showing the effect of HFD, Orlistat and herbal mix on serum cholesterol and triglyceride of high fat diet rats were represented in table (5)

The high fat diet rats exhibited a high significant increase of serum cholesterol and a significant increase in serum triglyceride as compared to normal rats.

The oral treatment of high fat diet with Orlistat exerted a high significant decrease in serum cholesterol and a significant decrease in serum triglyceride as compared to high fat diet rats.

The oral treatment of high fat diet rats with herbal mix extract showed a significant decrease in serum cholesterol and a significant decrease in serum triglyceride as compared to high fat diet rats (Table 5).

4- Effect of HFD, Zenical and herbal treatment on serum LDL and HDL in rats:

Data showing the effect of HFD, Orlistat and herbal mix on serum HDL and LDL of high fat diet rats were represented in table (5)

The high fat diet rats exhibited no significant increase of serum HDL and a high significant increase of serum LDL as compared to normal rats.

The oral treatment of high fat diet rats with Orlistat exerted a significant increase in serum HDL and a high significant decrease in serum LDL as compared to high fat diet rats.

The oral treatment of high fat diet rats with herbal mix exerted a significant increase in serum HDL and a high significant decrease in serum LDL as compared to high fat diet rats (Table 5).

5) Effect of HFD, Zenical and herbal treatment on kidney function tests:

Data showing the effect of HFD, Orlistat and herbal mix on urea, creatinine and uric acid of high fat diet rats were represented in table (6)

The high fat diet rats exhibited no significant increase of urea and a significant increase of creatinine, also showed no significant increase in uric acid as compared to normal rats.

The oral treatment of high fat diet rats with Orlistat exerted no significant decrease in urea and a significant decrease in creatinine, also showed no significant decrease in uric acid as compared to high fat diet rats.

The oral treatment of high fat diet rats with herbal mix exerted no significant decrease in urea and a significant decrease in creatinine, also it showed no significant decrease in uric acid as compared to high fat diet rats (Table 6).

6) Effect of HFD, Zenical and herbal treatment on renal oxidative stress:

Data showing the effect of HFD, Orlistat and herbal mix on renal GSH of high fat diet rats were represented in table (7)

The high fat diet rats exhibited a significant decrease of renal GSH and a high significant decrease of renal SOD while exhibited a high significant increase of renal MDA as compared to normal rats.

The oral treatment of high fat diet rats with Orlistat exerted a significant increase in renal GSH and a significant increase in renal SOD while a significant decrease in renal MDA as compared to high fat diet rats.

The oral treatment of high fat diet rats with herbal mix exerted a high significant increase in renal GSH and a significant increase in renal SOD while no significant decrease in renal MDA as compared to high fat diet rats (Table 7).

Light Microscopic Examination Of The Kidney:

Group (1) the kidney of control rats:

Histological examination of the normal kidney showed that it consist 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 that is a small tufts of fenestrated capillaries covered by thin diaphragm and Bowman's capsule (Fig. 2 a&b). The capsule's consists of an inner visceral layer and an outer parietal layer. The renal tubules consist of proximal convoluted tubules which are lined with cuboidal epithelium that have abrush border. The distal convoluted tubules are lined with low cuboidal epithelial cells, with no brush border, making their lumina appear wider.

Group (2) rats treated with high fat diet:

Light microscopic finding of kidney from high fat diet fed rats are summarized as follows dilated of blood vessels (Fig. 2) in addition to perivascular and periglomerular oedema infiltrated with a number of

inflammatory cells (Fig. 2d), degenerative change of renal tubules, vacuolated glomerular tuft surrounded with oedema and necrotic cells (Fig. 2 e) and enlargement of Bowman's space (Fig. 2 f).

Group (3) rats treated with high fat diet with Zenical:

The present light microscopical observations clearly demonstrated that administration of the Zenical to adult high fat diet rat induced various histological alternation in the kidney tissue. These changes included hypermic glomerular tuft associated with swelling in the lining epithelium of renal tubules, vacuolar glomerular tuft with swollen endothelial cell lining the Bowman's capsule (Fig. 3 g) and degenerated renal tubules and perivascular oedema with inflammatory cells (Fig. 3 h) while others showed blood vessel show severe congestion (Fig. 35i), focal necrotic area with inflammatory cells infiltration and some renal tubules suffer from degeneration(Fig. 3 j).

Group (4) rats treated with high fat diet with pumpkin oil +nutshell extract:

Animals treated with high fat diet plus pumpkin oil plus nutshell extract showed histological picture more or less similar to control group (Fig. 3 k) while others showed some haemorage between renal tubules (Fig. 3l).

Figure 2 :Photomicrograph of the kidneys sections of normal rats (a,b) and treated with high fat diet (c, d,e and f):

Fig (2 a&b): Photomicrograph of the kidneys sections of normal rats showing the glomerulus (G) and renal tubules (t). Fig (2a) H&E x 100 and Fig (2b) H&E x 400.

Fig (2 c): Photomicrograph of the kidneys sections of rats treated with high fat diet (HFD) showing a dilated blood vessels (↑) and perivascular oedema (O) and inflammatory cells (▲) infiltration. H & E x 400.

Fig (2 d): Photomicrograph of the kidneys sections of rats treated with high fat diet (HFD) showing perivascular and periglomerular oedema infiltrated with a number of inflammatory cells (↑). H & E x 400.

Fig (2 e): Photomicrograph of the kidneys sections of rats treated with high fat diet (HFD) showing degenerative change of renal tubules (RT), vacuolated glomerular tuft (v) surrounded with oedema (O) and necrotic cells (Nc). H & E x 400.

Fig (2 f): Photomicrograph of the kidneys sections of rats treated with high fat diet (HFD) showing expansion of Bowman's capsule (↑). H & E x 400.

Figure 3 :Photomicrograph of the kidneys sections of rats treated with high fat diet plus Zenical (g, H,I,j)and herbal extract (k, l):

Figs (3 g): Photomicrograph of the kidneys sections of rats treated with high fat diet plus Zenical 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 (3h): Photomicrograph of the kidneys sections of rats treated with high fat diet plus Zenical showing blood vessel hypermic with blood (Hy) and degenerated renal tubules (↑).H & E x 400.

Fig (3i): Photomicrograph of the kidneys sections of rats treated with high fat diet plus Zenical showing blood vessels show severe congestion (Hy) and intertubular hoamorrhage (↑).H & E x 400.

Fig (3j): Photomicrograph of the kidneys sections of rats treated with high fat diet plus Zenical showing focal necrotic area (↑) with inflammatory cells infiltration, blood vessels show congestion (Hy) and some renal tubules suffer from degeneration. H & E x 200.

Fig (3k): Photomicrograph of the kidneys sections of rats treated with high fat diet plus pumpkin oil plus nutshell extract showing nearly normal structure of glomerulus (G) and renal tubules (Rt). H & E x 400.

Fig (3l): Photomicrograph of the kidneys sections of rats treated with high fat diet plus pumpkin oil plus nutshell extract showing some hemorrhage between renal tubules (↑) and nearly normal structure of glomerulus (G) and renal tubules (Rt). H & E x 400.

Discussion

Obesity has become a heavy public health problem in the United States, with a prevalence among adults increasing to 32% from 13% between the 1960s and 2004 **(20)**. Currently, 66% of adults and 16% of children and adolescents are overweight or obese **(20)**. Although obesity has long been recognized as an independent risk factor for cardiovascular diseases and diabetes mellitus, newer research points to obesity as an important risk factor for chronic kidney diseases (CKDs) **(21)**. Massive obese patients developed nephrotic-range proteinuria. Subsequent studies confirmed that obesity could induce renal injury, namely, obesity-related glomerulopathy (ORG) **(22)**. A large-scale clinicopathologic study including 6818 renal biopsies from 1986 to 2000 revealed a progressive increase in biopsy incidence of ORG from 0.2% in 1986–1990 to 2.0% in 1996–2000. The tenfold increase in incidence of ORG over 15 years suggests a newly emerging epidemic **(23)**.

The mechanisms for how saturated fat and sugar-based beverages contribute to human obesity in rats on an HF choice diet, plasma leptin concentrations

and proopiomelanocortin mRNA increased and neuropeptide Y mRNA decreased (24).

Effect of control , HFD, Zenical and herbal mix on feed intake, body weight:

Dietary fat possesses a number of characteristics that may contribute to its overconsumption. Both palatability and energy density were contribute to fat hyperphagia and reduced satiation signaling accompanying HFD consumption which could contribute to overconsumption and often leads to obesity.

Belong to treatment with Zenical and herbal mix lead to decrease it due to blocking the absorption of fat by inhibiting gastric and pancreatic lipase enzymes leading to the increased excretion of fat in faeces (25).

On the other hand treatment with herbal mix lead to decrease body weight and feed intake because it 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 (26).

The obesogenic environment alters several inflammatory and metabolic effects and haemodynamic factors, which potentially lead to a decrease in renal functionality. A number of epidemiological studies demonstrated that inflammation is linked to metabolic syndrome in patients with CKD. A cross-sectional study of 94 CDK patients found an association between a high concentration of the inflammation marker CRP (> 3.0 mg/l) and BMI $\geq 30\text{kg/m}^2$ compared to patients with normal BMI (< 25 kg/m²) (27).

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

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 in obesity. **These results are in accordance with those of Szczygielska *et al.* (28).**

Patients receiving the two higher doses of Orlistat achieved significantly reduced levels of total cholesterol and low density lipoprotein (LDL-C). Low density lipoprotein-high density lipoprotein (LDL-HDL) ratio was significantly reduced in the highest dose Orlistat group compared to placebo. **(29).**

While 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 in agreement with that of **Moreno *et al.* (30) and Shifko (31)** they 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 low-density lipoproteins (LDL) cholesterol, lower triglycerides and higher high-density lipoproteins (HDL) cholesterol levels.

The reduction of the isoprenaline-stimulated lipolysis by PSE suggests that PSE may be taken inside the cells **Moreno *et al.* (32)**, where it decreases the activity of hormone sensitive lipase (HSL). Thus, the inhibition of HSL may

reduce levels of circulating free fatty acid (FFA) linked to insulin resistance in obese patients.

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

ORG is an increasing cause of end-stage renal disease (ESRD). The pathophysiology of ORG remains incompletely understood. 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. Increases in both glomerular filtration rate (GFR) and renal plasma flow (RPF) were observed in obese subjects and animals (33). This likely occurs because of afferent arteriolar dilation as a result of proximal salt reabsorption, coupled with efferent renal arteriolar vasoconstriction as a result of elevated angiotensin II (AngII) (34). These effects may contribute to hyperfiltration, glomerulomegaly and later focal glomerulosclerosis (23). Obese subjects usually have increases in plasma renin activity, angiotensinogen, angiotensin-converting enzyme activity and circulating AngII, which trigger or promote renal damage by renal hemodynamic changes and nonhemodynamic pathways such as hyperinsulinemia, oxidative stress and inflammation (35). Inflammatory abnormalities and oxidative stress are characteristic findings of obesity and play important roles in the renal damage associated with obesity.

High fat diet (HFD) caused 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 and nitric oxide inactivation by reactive oxygen species (ROS) and diminish NO bioavailability **(36)** which leading to renal dysfunction, characterizing by high level of creatinine and blood urea nitrogen **(37)**.

In obese subject, increased serum creatinine was to be observed suggesting that obesity caused elevation in renal function test and produce proteinuria, concomitantly with other risk factors such as hypertension, diabetes and dyslipidemia **(38)**. Obesity is recently acknowledged as an important independent risk factor for kidney disease. This risk is probably explained by renal intracellular lipid accumulation **(39)**. Also metabolic disorders in obesity associated with high blood pressure, poor glycemic control, dyslipidemia and smoking were considering risk factor for susceptibility of chronic renal disease (CRD). Glomerular hypertension and endothelial dysfunction were regarded as common mechanism of CRD rennin-angiotensin system. Renal triglyceride accumulated in obese rats accompanied by hypoalbuminemia and elevated blood urea nitrogen. Dysregulated gene expression may result in increased renal collagen cross-linking and lipid accumulation, that may be associated with development of nephropathy in the animal model of type 2 diabetes and obesity **(40)**. 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 **(41)**.

Obesity has long been recognized as an associated factor with a variety of adverse health consequences; chiefly among them diabetes, hypertension, dyslipidaemia, increased cardiovascular events and elevated serum uric acid **(42)**. Increases in serum uric acid concentration showed positive association with body mass index (BMI), waist hip ratio (WHR), Waist/thigh girth and

sub-scapula triceps skin fold ratios **(43)**. The risk of gout was increased among men who had been overweight in adolescence **(44)**. It has been suggested that other factors, such as muscle mass, may also play a role in producing high serum uric acid. Weight reduction has been associated with modest lowering of serum urate **(45)**.

Obese subjects had elevated serum uric acid approximately 10 times than non-obese subjects while overweight had elevated serum uric acid approximately 6 times than non-obese subjects.

Furthermore, serum uric acid was also positively correlated with serum triglyceride, LDL-C and negatively with HDL as have been found by **Facchini et al. (46)**. The association of obesity with overweight has a number of effects on urate metabolism, which include decreasing urate clearance and increasing urate production **(46)**.

Kidneys remove metabolic wastes such as urea, uric acid, creatinine and ions, so optimum chemical composition of body fluids is maintained. The concentrations of the metabolites increase in blood during renal diseases or renal damage may due to high activities of xanthine oxidase, lipid peroxidation and increased triacylglycerol and cholesterol levels **(47)**.

While treatment with Orlistat in our data showed that improvement in the level of renal function tests and decrease in kidney disease **(48)** who 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 **(49)** it was reported that Orlistat treatment was beneficial for patients to correct obesity because it improved metabolic processes and therefore kidney and liver functions.

Also, treatment with herbal mix (pumpkin oil) showed that decrease in renal function tests and improvement of kidney function **(50)** who observed that administration of flax/pumpkin or purslane/pumpkin seed mixtures showed announced significant decrease ($P < 0.05$) in the levels of serum urea, creatinine as compared with hypercholesterolemic control group (purslane or flax seeds were used as sources of ω -3 fatty acids). Flaxseed could potentially inhibit various mechanisms associated with the progression of renal diseases **(51)**. Flax and pumpkin seeds mixture rich in polyunsaturated fatty acid (PUFAs) and antioxidant compounds in animals.

Treatment with extract of peanut shell caused reduction in values of urea nitrogen and creatinine due to with active component luteolin that found in it **(52)**. There was 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 **(52)**.

While treatment high fat diet with Orlistat showed that improvement in the level of renal function tests and decrease in kidney disease **(48)**. It was found that the mechanism of action of gastrointestinal lipase inhibitors, the temporal association of accelerated renal function decline with the commencement of Orlistat, the high degree of compliance and weight reduction, the pathological findings and the absence of an alternative plausible explanation support our proposition that intrarenal precipitation of

calcium oxalate triggered the acute deterioration in kidney function in patients (48).

Also, treatment of high fat diet with herbal mix showed that decrease in renal function tests and improvement of kidney function (53). It was found that eating pumpkin seeds as a snack can help in preventing the most common type of kidney stone. Pumpkin seeds appear to both reduce levels of substances that promote stone formation in the urine and increase levels of compounds that inhibit stone formation. Some research had demonstrated that pumpkin seed oil (PSO) could remarkably reduce bladder pressure, increase bladder compliance and reduce urethral pressure (53).

Belong to treatment of high fat diet with peanut shell extract significantly improved renal function in rats with acute renal failure (ARF), significantly reduced renal oxidative damage (54). Peanut shell extract had a strong renal protective effect against glycerol-induced acute renal failure (ARF).

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

Dietary HFD causes 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 reactive oxygen species (ROS) and/or reduced antioxidant activity, leading to oxidative damage to cells or tissue including lipids, proteins and DNA. It is known that oxidative stress is involved in pathological processes of various diseases, such as cancer, diabetes mellitus, hypertension and cardiovascular disease. Studies have suggested that obesity is associated with increased oxidative stress (55).

Oxidative stress has been commonly identified in obesity-related renal diseases and may be the mechanism underlying the initiation or progression of renal injury in obesity **(56)**. Previous studies suggest that oxidative stress triggers, at an early age, the onset of kidney lesions and functional impairment in Zucker obese (ZO) fa/fa rats, a good model of obesity-related renal disease, in absence of hyperglycaemia, hypertension and inflammation **(57)**. ROS play a crucial role in mediating renal injury **(58)**. ROS are highly reactive molecules that oxidize lipids and proteins, cause cellular injury and promote glomerular and renal tubule injury and associated proteinuria **(59)**.

Oxidative stress also plays an important role in the pathogenesis of renal damage through its effects on vascular biology. ROS are generated by all types of vascular cells, including endothelial, smooth muscle and adventitial cells. ROS influence vascular cell growth, migration, proliferation and activation **(60)**. Physiologically, ROS can mediate cellular function, receptor signals and immune responses on vascular cells.

Our study indicated that treatment of HFD rats with Orlistat produced a significant increase in kidney GSH, a significant increase SOD and a high significant decrease in kidney MDA as compared to the drug-administrated-control rats.

Garcinia, this is drug similar in action to Orlistat in weighting loss significantly decreased levels of serum MDA and renal MDA compared with high fat diet (HFD) as result of hydroxy citric acid (HCA-SX) that inhibit ATP citrate lyase which catalyse extra mitochondrial cleavage of citrate to oxaloacetate giving acetyl COA which used in fatty acid synthesis, suggesting that Garcinia has hypolipidemic action and reduces MDA in kidney, so HCA improves lipid peroxidation **(61)**.

While treatment of HFD rats with herbal mix produced a significant increase in kidney GSH, a significant increase SOD and a high significant decrease in kidney MDA as compared to the high fat diet rats.

Wang *et al.* (54) reported that pretreatment with peanut shell extract (EPS) in acute renal failure (ARF) rats caused GSH in serum and renal cortical were observed significantly increased. Renal cortex homogenate MDA levels were significantly lower. And treatment with pumpkin oil that contain omega-3 caused the oxidative stress that had increased following reperfusion injury were also, significantly lowered by omega-3 administration **(62)**. Preventive oral administration of omega-3 supplement may decrease oxidative stress and kidney dysfunction following reperfusion injury.

Photomicrograph of the kidneys sections of normal HFD, Zenical and herbal treatment:

Obesity is *a chronic metabolic disorder that* results from the imbalance between energy intake and energy expenditure characterized by enlarged fat mass and elevated lipid concentration in blood. Many attempts have been made to correct *the metabolic disparity of the obesity condition, producing a number of* reagents including fibrates, sibutramine (an anorectic or appetite suppressant) and Orlistat but no drug is free from severe side effects **(63&64)**.

The present study demonstrates that a high-fat diet, fed for as little as 0 to 22 wk, (Figure 1) produced significant histologic, biochemical and functional changes in the kidney in comparison with normal group. These changes included expansion of Bowman's capsule **(65)**. The mechanisms responsible for the enlargement of Bowman's space in obesity are uncertain but may be related to the marked glomerular hyperfiltration; it is interesting

that the GFR was increased by almost the same percentage (38%) as the Bowman's space increase.

In our histological study, dilatation of glomerular capillaries and large blood vessels have been seen. In addition to tubular deformations, histologically detected glomerular atrophy, necrosis and oedema similar results were reported by **Altunkaynak *et al.* (66)**. The increase in the volume of the kidneys of HFD-fed rats may have resulted from oedema due to mononuclear cell infiltrations among the tubules. It is clearly understandable that dilatation may lead to a volumetric increase in the kidney. According to **Hvizdos and Markham (67)**, Orlistat is a novel non-systemic treatment for obesity it inhibits lipases in the gastrointestinal tract, preventing the absorption of approximately 30% of dietary fat. The present study revealed that the administration Zenical revealed that focal necrotic area with inflammatory cells infiltration and blood vessels show congestion and some renal tubules suffer from degeneration. Similar results were reported by **Korzets *et al.* (68)** who found that renal biopsy findings showed that the chronic kidney disease (CKD) was attributable to nephrocalcinosis/oxalate nephropathy, resulting in a chronic tubulo-interstitial nephropathy, while acute tubular necrosis. HFD increase serum uric acid (table 6) that initiate renal urate crystal producing histopathological changes.

Orlistat was capable of increasing urinary oxalate levels many-fold, especially in rats fed high fat or oxalate-enriched diet. A rise in the ion-activity product of calcium oxalate was also demonstrated. Interestingly, no renal depositions of oxalate were seen on examination of 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 **(69)**. Also, in 2008, **Karamadoukis *et al.* (70)** 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 **(71)**.

HFD increase uric acid that elevate urinary acidity which increase the renal histological changes while treatment with pumpkin seed oil decrease renal acidity (toward alkalinity) due to its high phosphorus content so renal histological changes have been improved.

It could be concluded that rats fed HFD showed, hypertriglyceridemia, increased LDL levels, increased oxidative stress and renal histopathological alteration. Moreover, suggesting association between lipid peroxidation, obesity and nephropathy, while treatment with herbal mixture ameliorated the harmful effects of the HFD and reduce feed intake, and improve oxidative stress in renal tissues in comparison with orlistat.

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