



Original Article

Effects of Virgin Olive Oil on Renal Function and Histopathological Alterations in Hyperlipidemic Rats

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Abstract

Background: hyperlipidemias represent one of the possible factors which contribute to the aetiology, pathogenesis and establishment of renal diseases. In our present work, we are targeting to study the effect of the administration of Virgin Olive Oil at low and high doses on kidney function and histopathology in hyperlipidaemic male albino rats. **Material & Methods:** Male albino rats were grouped into a normal and hyperlipidemic diet, and some hyperlipidemic groups were treated with the addition of virgin olive oil at (LOW and HIGH doses). Serum kidney parameters were measured to evaluate kidney functionality. Kidneys were then excised, processed and examined for histology by staining with Hematoxylin and Eosin (H&E). **Results:** In hyperlipidemic treated animals (LOW and HIGH doses), the levels of urea concentration were significantly reduced in the two groups as compared to the other two groups (hyperlipidemic without treatment and normolipidemic). No significant differences were observed for serum creatinine levels between any of the experimental groups. Light microscopic examination of the kidneys revealed evident restoration of the kidney tissue lesions that were present in the hyperlipidemic without treatment groups, and a greater recovery effect was reported in the high dose of olive oil, with kidney tissues close to the normalised kidney tissues. **Conclusion:** virgin olive oil, at both doses (LOW and HIGH), protects against kidney injury resulting from hyperlipidemias in male albino rats. It improves renal tissue histopathology and lowers serum urea concentration in conditions of hyperlipidemias.

Keywords: Virgin olive oil; hyperlipidemia; Kidney injury; histopathology; rats.

Introduction

Evidence shows that improper lipid metabolism leads to kidney damage, which worsens chronic kidney disease (CKD) progression [1,2]. Researchers must study how hyperlipidemia affects kidney function to develop better ways to treat and prevent kidney disease.

The link between dyslipidemia and decreased kidney function has been established through multiple epidemiological studies [3,4]. Elevated triglycerides and low high-density lipoprotein (HDL) levels are independently associated with increased risk of incident CKD [3]. The study found that people with higher total cholesterol levels experienced a decline in their estimated glomerular filtration rate (eGFR) [5]. The high prevalence of dyslipidemia among CKD patients indicates a two-way connection between these conditions [6].

Clinical studies demonstrate that high levels of LDL cholesterol and triglycerides lead to faster CKD progression and higher ESRD risk [4,5]. Hyperlipidemia in diabetic nephropathy worsens both proteinuria and renal damage [7]. The renal protective effects of statins as lipid-lowering therapies exist at a modest level because they

reduce inflammation and lower lipid levels [8,9]. The treatment of hyperlipidemia has the potential to slow down the progression of kidney disease [8,5]. Statins and fibrates enhance lipid profiles while potentially decreasing albuminuria levels [8]. The direct benefits of lipid-lowering therapy on long-term kidney health increase the need for study despite its established capacity to lower cardiovascular event rates [9].

The present study was designed to investigate the influence of low and high doses of virgin olive oil administration to normal and hyperlipidaemic male albino rats on kidney function. At the end of the experiment period, rats were sacrificed for organ sample collection and histopathological examination.

Materials and methods:

Animals and Housing:

The study was conducted in the Animal House of the National Research Centre (NRC), Cairo, Egypt. From August to November 2016. Seventy male Sprague Dawley rats (90-110 g) were randomly selected. All rats were active, apparently healthy and free from abnormalities and



disease. The experimental animals were housed in commercial cages, equipped with automatic drinkers and feeders. They were supplied with food and water ad libitum under standard conditions of light (14-10) light-dark cycle, humidity and temperature (22-25°C). All experimental procedures in this study were carried out according to the guidelines of the Ethics Committee of the NRC, Cairo, Egypt.

Feeding regimen:

Basal and experimental diets were formulated to cover the requirements of growing rats as recommended in NRC (1977). Composition and proximal chemical analysis of formulated diets are shown in Table 1. Diets were subjected to chemical analysis according to AOAC (2012).

Olive Oil:

Olive oil in the present study was obtained from olive (*Olea europaea*; family Oleaceae), a traditional tree crop of Tarhuna city farms, according to the Agriculture Research Centre – Tarhuna – Libya (Bartolini *et al.*, 1998). Olive oil was administered in two doses:

- Low dose (1 ml / 100g B.W) olive oil.
- High dose (2 ml / 100g B.W) olive oil.

Table 1: The Composition of the Diet

Ingredient (g/kg)	Basal diet	Exp. Diet
Corn	729.60	729.60
Casein	17.56	17.56
Fat	2.7	2.7g/100g
Butter oil	0.00	19g
Soy bean oil	0.00	1.00g
Mineral/vitamin mix	60.40	60.40

Table 2: Proximal analysis of basal diet.

Item	%
Moisture	46.67
Ash	3.17
Crude protein	13.76
Crude fibre	2.15
Crude Fat	8.43

Table 3: Fatty acid composition of dietary olive oil (g/100 g)

Fatty acid	g/100 g
16:0	11.32
16:1 (w-9)	0.11
16:1 (w-7)	0.84
18:0	4.34
18:1 (w-9)	74.12
18:2 (w-6)	7.64
18:3 (w-3)	0.61

Induction of hyperlipidaemia:

Hyperlipidaemia was induced by feeding rats for three weeks with a mixture composed of 20g of fat / 100g of diet (19 g of butter oil and 1 g of soybean oil) to provide essential fatty acids, added to basal diets, according to the method described by Stephen *et al.* (2003). Serum samples were collected to confirm hyperlipidaemia in groups receiving fat in the diet.

Experimental design:

Two experiments were performed after the adaptation period.

1st experiment:

The rats were equally and randomly divided into five groups (10 in each):

The first group was considered the control group, received the Basel diet (ND) and was supplemented with 1ml saline by gastric tube for 4 weeks. The second group received diet-induced hyperlipidaemia (HLD) until the end of the experiment (4 weeks). The third group received diet-induced hyperlipidaemia and was supplemented with a low dose of virgin olive oil (1 ml/100g B.W) (HLD+LVOO), administered by gastric tube for 4 weeks. The fourth group received diet-induced hyperlipidemia and was supplemented with a high dose of virgin olive oil (2 ml/100g B.W) (HLD+HVOO), administered by gastric tube for 4 weeks. The fifth group, after receiving diet-induced hyperlipidemia, followed the Basel diet (HLD +ND) to the end of the experiment.

experiment:

The rats were equally and randomly divided into three groups (10 in each):

The first group was considered the control group, received the Basel diet and was supplemented with 1ml saline by gastric tube for 4 weeks. The second and third group were received the Basel diet, and were supplemented with 1ml/100g and 2ml/100g of virgin olive oil (VOO), respectively, administered by gastric tube for 4 weeks.

Blood samples were collected individually by the orbital venous plexus technique under mild ether inhalation anaesthesia. Samples were obtained in the early morning before access to feed and water at the end of every week. Blood samples were collected into plain tubes and allowed to coagulate at room temperature, and centrifuged at 1000 g for 20 min to obtain sera. The clear, non-haemolysed supernatant sera was quickly collected for each animal and stored at (-20 °C) until used for determination of biochemical assay.

Organs: At the end of the experimentation period, rats were sacrificed for organ sample collection per replicate.

**Kidney function test:****Determination of Urea (mg/dl):**

Urea level was determined using the enzymatic colourimetric method of Fawcett and Scott (1960) by a kit obtained from Biodiagnostic Egypt.

Determination of Creatinine (mg/dl):

Creatinine was determined in serum using commercial kits purchased from Biodiagnostic Egypt, according to the methods of Houot (1985).

Histopathological examination:

At the end of the experiment, the animals were euthanised, and samples of liver, kidney and heart were preserved on formaline 10 % for histopathological examination. Samples were stained with hematoxylin and eosin for microscopic observations (Bancroft and Stevens, 1997).

Results

Results presented in Table (1) and Figure (1) showed hyperlipidemia (HLD) induced a significant ($P \leq 0.05$) increase in levels of serum urea during the 1st, 2nd, 3rd, and

4th weeks of the experimental periods (46.0 ± 2.23 , 53.0 ± 2.73 , 58.0 ± 1.58 , and 62.0 ± 2.34 mg/dl), as

compared with normal diet (ND) group (41.00 ± 2.62 , 42.00 ± 1.58 , 44.00 ± 2.00 , and 45.00 ± 2.73 mg/dl), respectively.

There was no significant difference in levels of serum **creatinine** during the 1st, 2nd, 3rd, and 4th weeks of the experimental periods between all groups, as compared to the control group receiving a normal diet only during the experimental periods.

Moreover, groups subjected to a hyperlipidemic diet and supplemented with a low dose of virgin olive oil (HLD + LVOO) or a high dose of virgin olive oil (HLD + HVOO) revealed a significant ($P \leq 0.05$) decrease in serum urea levels, as compared with both the hyperlipidemic diet group and the normal diet (ND) group. Furthermore, the group subjected to a hyperlipidemic diet and supplemented with a normal diet (HLD + nd) showed non-significant ($p \leq 0.05$) serum urea and creatinine levels, as compared with other groups.

Table 4: Effect of olive oil administration on kidney function test in hyperlipidemic rats.

Groups Param eters		ND	HLD	HLD+LVOO	HLD+HVOO	HLD+ND	LSD
Urea (mg/dl)	I	41.00± 1.62	46.0± 1.23	45.0± 1.58	43.0± 1.58	44.0± 1.58	2.68
	II	42.00 ±1.58	53.0± 1.73	44.0± 1.00	41.0±1.12	43.0± 2.54	
	III	44.00± 1.00	58.0±1.58	42.0± 1.87	40.0± 1.23	43.0± 2.12	
	IV	45.00± 1.73	62.0± 1.34	42.0± 1.53	39.0± 1.58	42.0± 1.58	
Creatinine (mg/dl)	I	1.67± 0.02	1.65± 0.02	1.63± 0.02	1.67±0.01	1.66± 0.01	N.S
	II	1.69± 0.01	1.68± 0.02	1.66± 0.01	1.69± 0.03	1.67± 0.01	
	III	1.72±0.01	1.67± 0.02	1.73±0.01	1.70± 0.02	1.67± 0.01	
	IV	1.73±0.02	1.71±0.01	1.70± 0.01	1.71±0.02	1.70± 0.01	
ANOVA		P≤ 0.05					

Data indicate mean ± standard error at ($p \leq 0.05$), N= 10 rats, ND= Control, HLD= Hyperlipidemic diet, HLD+LVOO

= Hyperlipidemic diet + low dose of virgin olive oil, HLD+HVOO = Hyperlipidemic diet + high dose of virgin olive oil, HLD+ND= Hyperlipidemic diet followed by normal diet, I= 1st week, II= 2nd week, III= 3rd week, IV= 4th week, LSD= (Least significant difference).

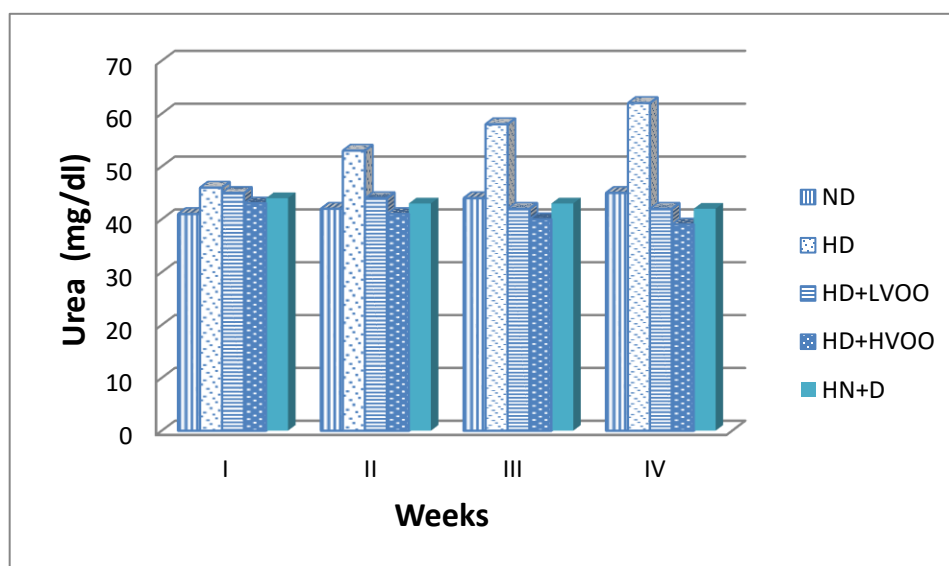


Figure 1: Effect of olive oil administration on urea in the serum of hyperlipidaemic rats.

ND= Control, HLD= Hyperlipidemic diet, HLD+LVOO = Hyperlipidemic diet + low dose of virgin olive oil, HLD+HVOO= Hyperlipidemic diet + high dose of virgin olive oil, HLD+ND= Hyperlipidemic diet followed by normal diet.

Histopathology

Rats received a normal diet (ND) group showed normal renal glomeruli and renal tubules (Fig.2.a), while the kidneys in rats subjected to a hyperlipidemic diet (HLD) group showed vacuolation of the renal tubular epithelium, cellular swelling and fatty degeneration of most renal tubules. Dilated congested vessels were prominent with

many areas of interstitial haemorrhage and perivascular oedema (Fig.2.b). Moreover, the kidney section in rats subjected to a hyperlipidemic diet and supplemented with low-dose virgin olive oil (HLD+LVOO) group (Fig.2.c) shows a markedly normal redistribution pattern of perivascular oedema of the interstitial blood vessel (arrow). Furthermore, the kidney section in rats subjected to a hyperlipidemic diet and supplemented with high dose virgin olive oil (HLD+HVOO) (Fig.2.d) showed nearly normal structures of the renal tubules and glomeruli, a reduction in perivascular oedema, and vacuolation of the renal tubular epithelium (arrow).

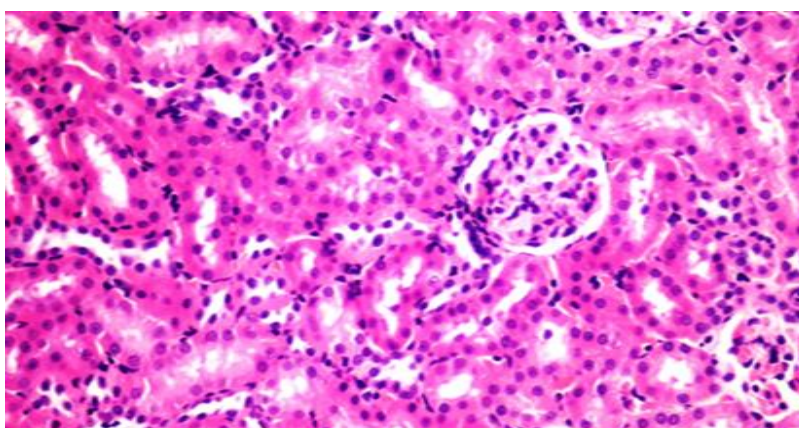


Figure 2a- Kidneys showing apparently normal renal glomeruli and renal tubules (H&E X 400).

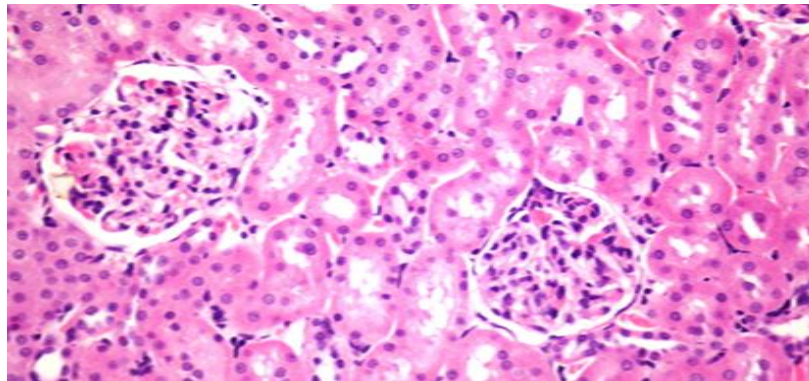


Figure 2 b- Kidneys showing vacuolation of the renal tubular epithelium (arrow) (H&E X 400).

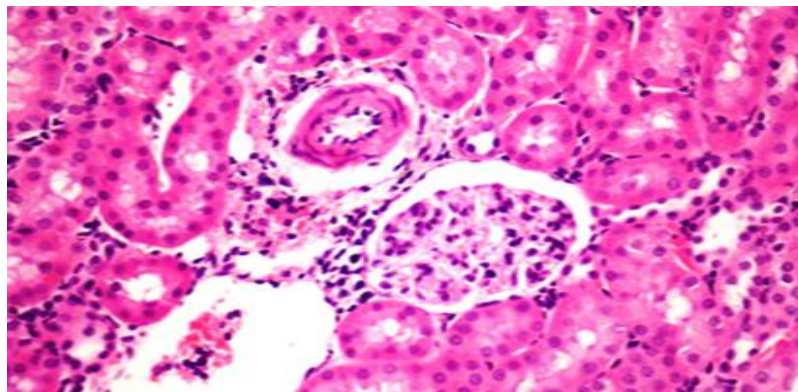


Figure 2c- Kidneys showing perivascular oedema of the interstitial blood vessel (arrow) (H&EX 400).

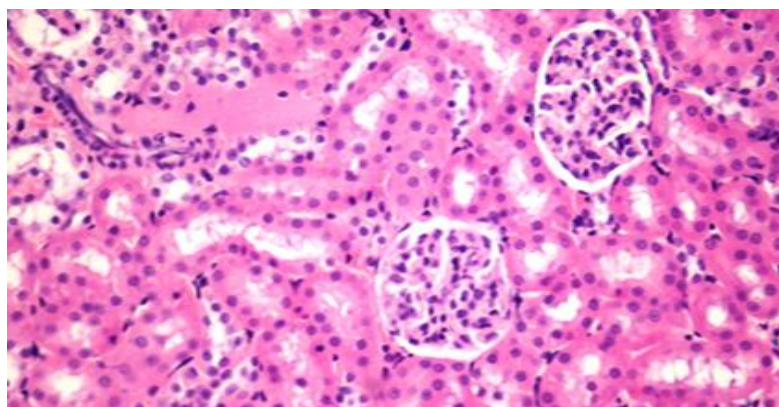


Figure 2 d- Kidneys showing reduction in perivascular oedema and vacuolation of the renal tubular epithelium (arrows. (arrow) (H&E X 400).

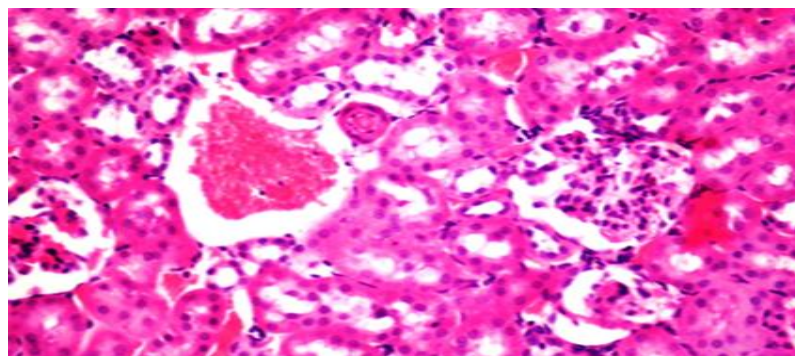


Figure 2 e- Kidneys showing congestion in the interstitial blood vessel (arrow) (H&E X 400).

Discussion

The obtained results revealed a significant increase in the values of serum urea levels in rats subjected to diet-induced hyperlipidemic (HLD). Nevertheless, creatinine levels did not show any significant variations, as compared with the control value during the 1st, 2nd, 3rd, and 4th weeks of the experiment. Hyperlipidemia may play a role in the pathogenesis of renal diseases through the enhancement of protein catabolism and acceleration of amino acid deamination for gluconeogenesis; an acceptable postulate to interpret the elevated levels of serum urea. Further reports showed that hyperlipidemia induces glomerular injury, which was indicated by increased serum urea level [17,18],[19,20], [21,22].

Rats fed a hyperlipidemic diet and supplemented with a low dose of virgin olive oil or a high dose of virgin olive oil showed a significant improvement in serum urea levels. Similarly, previous results found that virgin olive oil elevated serum total proteins, albumin, and globulin levels. [23,24]. Rats subjected to a hyperlipidemic diet and supplemented with a normal diet showed no significant difference in their performance, as compared with other groups. Indicating that olive oil has a beneficial effect on these parameters.

The histopathological results obtained in this study on rats fed a hyperlipidemic diet were revealed by other studies [23,24]. which explained that excessive lipid accumulation promotes the generation of reactive oxygen species (ROS)

and lipid peroxidation, which results in renal tissue damage and kidney function impairments (27).

The tissue of animals fed with high doses of virgin olive oil showed greater improvements than other groups studied. Histological examination revealed normal renal tubules and glomeruli with reduced vacuolation and perivascular oedema. Similar results were obtained by previous studies, which explained that olive oil supplementation improves both kidney morphology and biochemical parameters (29) and reduces the production of inflammatory mediators in kidney tissue, which leads to decreased tissue damage (30).

Conclusion

Virgin olive oil supplementation protected remarkably the hyperlipidemia-induced renal injury in male albino rats through a significant decreased serum urea concentration and excellent recovery of renal histological damage with an unchanged serum creatinine concentration during the experimental period. The higher dose was more effective in improving than the lower dose of virgin olive oil, and the renal tissue displayed nearly normal histological structure. These results suggest that virgin olive oil could be considered a dietary preventive approach to reduce hyperlipidemia-related renal injury, and additional studies are needed to elucidate the molecular mechanisms responsible for virgin olive oil-induced renoprotective effects.

Conflict of Interest

There is no financial, personal, or professional conflict of interest regarding the publication of this study.

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