

Modulation of blood cholesterol in hypercholesterolemic male rats by dietary administration of either fenugreek (*Trigonella foenum-graecum*) leaves or seeds

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Abstract: The leaves or seeds powder of fenugreek (*Trigonella foenum-graecum*) were orally administered in the diet to hypercholesterolemic male rats for 8 weeks to test their effect on lipid profile, antioxidant enzymes and lipid peroxide. Twenty four male rats weighing 150-170 gm were divided into four groups. The first group is untreated (control group), fed basal diet, the second group, fed on 2% cholesterol in diet to induce hypercholesterolemia (positive control group), the third and the fourth group fed 2% cholesterol (to induce hypercholesterolemia) and treated with 500 mg/kg body weight fenugreek leaves or seeds, respectively for 8 weeks. The positive control group showed a significant increase in lipid profile, liver enzyme, lipid peroxide and kidney function parameters, and serum electrolytes and decrease in antioxidant enzymes activity. In addition, heart, liver, kidney and testes showed histopathological changes compared with the negative control. Treating the hypercholesterolemic rats with fenugreek leaves or seeds improved the biochemical blood tests and the histology of the studied organs tissues and restored them to the normal state. In conclusion, fenugreek leaves and fenugreek seeds have an anti-oxidant activity and ameliorated the hyperlipidemia, improved liver and kidney functions and decreased lipid peroxide in hypercholesterolemic male rats. The antihyperlipidemic activity of fenugreek could be attributed to inhibiting oxidative stress.

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1. Introduction

Fenugreek (*Trigonella foenum-graecum*) belongs to the family *Fabaceae*. It is cultivated worldwide for its seeds that used as an important ingredient in dishes. The seeds of fenugreek plant are widely used in the preparation of seasonings, pickles, curry powders and dietary supplements (Liu et al., 2012). Chatterjee and Sharma (2010) found that triacylglycerol and phosphatidylethanolamine were the major molecular species identified in the neutral and polar lipid fractions of fenugreek seeds, respectively.

Hyperlipidemia has been shown to be a strong risk factor for coronary heart diseases indication and risk factor for early atherosclerosis prior to the appearance of over atherosclerotic changes in the vascular wall (Bentley et al., 2002 and Makni et al., 2008). Clinical trials have shown that lowering lipids reduces the morbidity and mortality associated with cardiovascular complication (Amundsen et al., 2002).

Lipid profile could be lowered using food supplementation in the diet (El Rabey et al., 2013). The high levels of lipids (cholesterol, HDL, LDL and triglycerides) and uric acid in the diabetic rats were restored to levels found in non-diabetic controls by the treatment with 4HO-Ile of fenugreek (Haeri et al., 2012). The contents of thiobarbituric acid-reactive substances (TBARS), catalase and superoxide dismutase (SOD) in liver, heart and kidney were

decreased after oral administration of the ethyl acetate fenugreek extract fed rats (Belguith-Hadriche et al., 2013).

Previous studies showed that fenugreek has also an antidiabetic activity (Kumar et al., 2005 and Xue et al., 2011). It ameliorates diabetic hypertensive nephropathy by suppression of oxidative stress in kidney and reduction in renal cell apoptosis and fibrosis in streptozotocin induced neonatal diabetic rats (Ghule et al., 2012; Sindhu et al., 2012 and Kenny et al., 2013). Kaviarasan et al. (2007) evaluated the antioxidant activity of fenugreek methanol extract using various in vitro assay systems and referred that fenugreek seed extract exhibited scavenging of hydroxyl radicals ($\cdot\text{OH}$) and inhibition of hydrogen peroxide-induced lipid per-oxidation in rat liver mitochondria. Dietary administration of (1 and 2%) fenugreek seeds resulted in an increase of GSH and the glutathione S-transferase (GST) activities in the liver homogenate of rats and have no appreciable change in superoxide dismutase (SOD) and catalase (Choudhary et al., 2001). Furthermore, the hydro-alcoholic extract of fenugreek ameliorated various impairments associated with physical fatigue in rats subjected to weight loaded forced swim test (Kumar et al., 2013). In addition, fenugreek as spice in functional food exhibited anti-inflammatory and antioxidant activities (Liu, et al., 2012).

The aim of this study was testing the effect of treating hypercholesterolemic male rats with either fenugreek leaves or seeds for 8 weeks to test their effect on lipid profile, antioxidants enzymes and lipid peroxide.

2. Materials and Methods

Fenugreek leaves were collected from fresh growing fenugreek plants, dried at room temperature and milled by mixer then mixed to the diet in a ratio of 500 mg/kg body weight. Fenugreek seeds were obtained from local agricultural company, milled by mixer and then mixed to the diet in a ratio of 500 mg/kg body weight.

Basal lipid rich diet:

The basal diets consisted of the following constituents: 16% casein, 10% corn oil, 4% N.N cellulose, 4% Salt Mixture, 1% Vitamin Mixture, 0.2% choline chloride, 0.2% DL. methionine and 64.5% Corn starch.

Animals and housing

All the animal experiments were carried out under protocols approved by the Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia. Twenty four male rats "*Rattas rattas*" weighing 150-170g were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were housed six per polycarbonate cages. Cages, bedding, and glass water bottles (equipped with stainless steel sipper tubes) were replaced twice per week. The stainless steel feed containers were changed once per week.

Experiment design

The animals were kept at room temperature ($25 \pm 5^\circ\text{C}$) with a natural lighting cycle (12 hours), fed a standard basal diet and kept under observation for 2-weeks before the experiment starts to exclude any undercurrent infection. The test animals were then divided randomly into four groups as follows: first group is untreated (control group) was fed on normal diet, the second group was fed on 2% cholesterol (Onody et al., 2003 and Rathod et al., 2011) in diet to induce hypercholesterolemia (positive control group), the third and the fourth groups were fed on 2% cholesterol (to induce hypercholesterolemia) and treated with 500 mg/kg body weight fenugreek leaf powder and fenugreek seed powder for 8 weeks. The current study was continued for 8 weeks to induce hypercholesterolemia (Ahmed, 2001 and El Rabey et al., 2013). At the end of the experiment, animals were fasted 14-16 hours after their last feeding and blood samples were collected from the

heart of pre-anaesthetized rats (anaesthetized by Dimethyl-ether). Blood was collected in plain tubes for biochemical analyses. Blood serum was obtained by centrifugation at 1000 rpm for 10 min at room temperature, and then stored at -20°C until analysis.

Biochemical tests

After collection of blood, anaesthetized animals were scarified by cervical dislocation. The abdomen was opened and the organs were rapidly dissected out and weighed. Apiece of liver was saved in ice-cold for antioxidant enzyme.

The following parameters were estimated:

i- Serum lipids:

Serum total cholesterol (S.TC), serum triglyceride (S.TG), serum high density lipoprotein cholesterol, low density lipoprotein cholesterol (S.LDLc) and serum very low density lipoproteins cholesterol (VLDLc) were estimated using Human kit (Germany).

ii- Liver enzymes:

Serum alanine aminotransferase (ALT), serum Gamma-glutamyl transferase (γ -GT) and serum alkaline phosphatase (ALP) were estimated using Human Kit (Germany).

iii- Antioxidants enzymes:

Serum catalase, serum glutathione reductase (GR) and serum superoxide dismutase (SOD). These three antioxidant enzymes were estimated in liver homogenate using the specified kits from Biodiagnostic Chemical Company (Egypt) according to the instructions of the suppliers.

iv- Lipid peroxide:

Malondialdehyde (MDA) was estimated in kidney homogenate were estimated in liver homogenate using the specified kits from Biodiagnostic Chemical Company (Egypt) according to the instructions of the suppliers.

v- Kidney functions:

Serum uric acid, serum creatinine and serum urea were estimated using Human Kit (Germany).

vi- Serum electrolytes:

Calcium, sodium, phosphorus and potassium ions were estimated in serum using the specified kit from Human (Germany) according to the instructions of the suppliers.

Histopathological investigations

Target organs; liver, heart, right kidney and testes were washed in sterile saline and fixed in 10% neutral formalin for histopathological studies. Organs were dehydrated in gradual ethanol (50-99%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H&E) dye for microscopic investigation (Drury et

al., 1976). The stained sections were examined and photographed under a light microscope.

Statistical analysis

All data were analyzed using the SPSS (Statistical Program for Sociology Scientists) Statistics Version 17.0 for computing the mean values, the standard errors (SE) and test of significance (t-test).

3. Results

3.1. Lipid profile

Table (1) shows the effect of either fenugreek leaves or seeds on serum lipids in hypercholesterolemic rats for 8 weeks. As shown the value of TC (Total Cholesterol) of the positive control group was very high significantly (at $P<0.001$) higher than that of the negative control group (128.35 ± 1.22 and 100.27 ± 4.99 mg%, respectively). Treating the hypercholesterolemic rats with fenugreek leaves and seeds has significantly (at $P<0.05$) lowered the STC than that of the positive control (109.02 ± 9.35 , 114.67 ± 5.10 and 128.35 ± 1.22 mg%, respectively).

Regarding triglyceride (TG), the mean value of TG in the positive control group was very high significantly (at $P<0.001$) higher than that of the fenugreek leaves treated group, fenugreek seeds treated group and that of the negative control group (117.07 ± 6.56 , 53.06 ± 8.44 , 52.71 ± 6.79 and 81.41 ± 1.60 mg/dl, respectively).

The mean value of serum high density lipoprotein cholesterol (S.HDLc) of fenugreek leaves and seeds treated groups were very high significantly (at $P<0.001$) higher than that of the positive control (55.03 ± 0.43 , 53.86 ± 0.82 and 40.16 ± 1.49 mg/dl, respectively).

Concerning serum low density lipoprotein cholesterol (S.LDLc), the mean values of fenugreek leaves and seeds treated groups were nonsignificantly more or less than that of the control groups. On the other hand, for the serum very low density lipoprotein cholesterol (S.VLDLc), the mean values of negative control, fenugreek leaves and seeds treated groups were very high significantly less (at $P<0.001$) lower than that of the positive control (16.28 ± 0.32 , 10.61 ± 1.68 , 10.54 ± 1.35 and 23.32 ± 1.30 mg/dl, respectively).

Table (1): Effect of treating hypercholesterolemic rats with either fenugreek leaves or seeds for 8 weeks on serum lipids.

Parameters	Treatments	G1	G2	G3	G4
	Statistics	(-)ve Control	(+)ve Control	Fenugreek leaves	Fenugreek seeds
S.TC (mg %)	Mean \pm SE	100.27 \pm 4.99	128.35 \pm 1.22	109.02 \pm 9.35	114.67 \pm 5.10
	T. test		-5.36***	1.99*	2.45*
S.TG mg/dl	Mean \pm SE	81.41 \pm 1.60	117.07 \pm 6.56	53.06 \pm 8.44	52.71 \pm 6.79
	T. test		-5.09***	6.34***	5.48***
S.HDLc mg/dl	Mean \pm SE	42.70 \pm 3.13	40.16 \pm 1.49	55.03 \pm 0.43	53.86 \pm 0.82
	T. test		0.66 ^{NS}	-10.06***	-8.96***
S.LDLc mg/dl	Mean \pm SE	43.66 \pm 2.47	46.35 \pm 1.00	43.52 \pm 8.54	43.50 \pm 5.21
	T. test		-0.79 ^{NS}	0.34 ^{NS}	0.52 ^{NS}
S.VLDLc mg/dl	Mean \pm SE	16.28 \pm 0.32	23.32 \pm 1.30	10.61 \pm 1.68	10.54 \pm 1.35
	T. test		-5.06***	6.17***	5.46***

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant * $P<0.05$ *** $P<0.001$.

G1: Control (-)ve: Normal rats fed on basal diet.

G2: Control (+)ve: Rats treated with 2% cholesterol and fed on basal diet.

G3: Rats treated with 2% cholesterol and treated with fenugreek leaves.

G4: Rats treated with 2% cholesterol and treated with fenugreek seeds.

3.2. Serum liver enzymes

Table (2) shows the effect of either fenugreek leaves or seeds treatment on serum liver enzymes in hypercholesterolemic rats for 8 weeks. As shown, the mean value of serum alanine aminotransferase (ALT) of negative control group, fenugreek leaves treated group and fenugreek seeds treated group were high significantly (at $P<0.01$) lower than that of the positive control group (23.61 ± 1.59 , 28.13 ± 3.77 , 41.50 ± 5.24 and 29.80 ± 3.88 U/l, respectively).

For serum Gamma-glutamyl transferase (S. γ -GT), all the mean values of the negative control

group, fenugreek leaves treated group and fenugreek seeds treated group were nonsignificantly less than that of the positive control group.

As shown, the mean values of serum alkaline phosphatase (S.ALP) of the negative control and fenugreek leaves treated groups were significantly (at $P<0.05$) lower than that of the positive control (61.78 ± 13.36 , 68.55 ± 6.81 and 91.66 ± 3.98 U/l, respectively), whereas the mean value of S.ALP in the fenugreek treated group was non significantly lower than that of the positive control group (81.60 ± 4.87 and 91.66 ± 3.98 U/l, respectively).

3.3. Antioxidants enzymes

Table (3) shows the effect of either fenugreek leaves or seeds treatment on antioxidants enzymes in the liver tissue homogenate of hypercholesterolemic rats for 8 weeks. As shown in Table (4), the mean value of catalase in the negative control group, fenugreek leaves treated group and fenugreek seeds treated groups were very high significantly (at $P<0.001$) higher than that of the positive control (258.50±15.65, 136.00±3.55, 126.50±2.23 and 113.00±2.76mmol/g tissue, respectively).

For superoxide dismutase, the mean value of the positive control group was non significantly lower

than that of the fenugreek leaves treated group, the fenugreek treated group and the negative control group (245.50±29.01, 274.68±6.93, 254.52±21.93 and 262.63±16.58 mmol/g tissue, respectively).

The mean value of glutathione reductase in the liver tissue homogenate of the positive control group was very high significantly (at $P<0.01$) lower than that of fenugreek leaves treated group, fenugreek seeds treated group and the negative control group (7.91±0.55, 13.90±0.58, 14.78±0.39 and 16.98±0.69mmol/g tissue, respectively).

Table (2): Effect of treating hypercholesterolemic rats with either fenugreek leaves or seeds for 8 weeks on serum liver enzymes.

Parameters	Treatments Statistics	G1 (-)ve Control	G2 (+)ve Control	G3 Fenugreek leaves	G4 Fenugreek seeds
S.ALT U/L)	Mean±SE	23.61±1.59	41.50±5.24	28.13±3.77	29.80±3.88
	T.test		-3.49**	2.95**	1.98**
S. γ-GT U/L)	Mean±SE	3.75±0.18	4.28±0.47	4.20±0.27	4.25±0.11
	T.test		-0.94 ^{NS}	0.13 ^{NS}	0.07 ^{NS}
S.ALP U/L)	Mean±SE	61.78±13.36	91.66±3.98	68.55±6.81	81.60±4.87
	T.test		-2.16*	2.47*	1.59 ^{NS}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant * $P<0.05$ ** $P<0.01$.

G1: Control (-)ve: Normal rats fed on basal diet. G2: Control (+)ve: Rats treated with 2% cholesterol and fed on basal diet.

G3: Rats treated with 2% cholesterol and treated with fenugreek leaves.

G4: Rats treated with 2% cholesterol and treated with fenugreek seeds.

Table (3): Effect of treating hypercholesterolemic rats with either fenugreek leaves or seeds for 8 weeks on serum antioxidants enzymes.

Parameter	Treatments Statistics	G1 (-)ve Control	G2 (+)ve Control	G3 Fenugreek leaves	G4 Fenugreek seeds
Catalase mmol/g tissue	Mean±SE	258.50±15.65	113.00±2.768	136.00±3.55	126.50±2.23
	T. test		10.855***	10.855***	-6.935***
Superoxide dismutase mmol/g tissue	Mean±SE	262.63±16.58	245.50±29.01	274.68±6.93	254.52±21.93
	T. test		0.53 ^{NS}	-0.93 ^{NS}	-0.21 ^{NS}
Glutathione reductase mmol/g tissue	Mean±SE	16.98±0.69	7.91±0.55	13.90±0.58	14.78±0.39
	T. test		8.79***	2.72***	2.78***

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant ** $P<0.01$ *** $P<0.001$.

G1: Control (-)ve: Normal rats fed on basal diet.

G2: Control (+)ve: Rats treated with 2% cholesterol and fed on basal diet.

G3: Rats treated with 2% cholesterol and treated with fenugreek leaves.

G4: Rats treated with 2% cholesterol and treated with fenugreek seeds.

3.4. Lipid peroxide

Table (4) shows the effect of either fenugreek leaves or seeds treatment on lipid peroxide in the liver tissue homogenate of hypercholesterolemic rats for 8 weeks. As shown in figure (5), The fenugreek leaves and seeds treatment reduced the lipid peroxide as revealed from the mean value of lipid peroxide of

the positive control group that was very high significantly (at $P<0.001$) higher than that of the fenugreek leaves treated group, fenugreek seeds treated group and that of the negative control group (792.33±31.28, 652.52±23.91, 562.67±37.28 and 626.77±28.05 nmol / g tissue, respectively).

Table (4): Effect of treating hypercholesterolemic rats with either fenugreek leaves or seeds for 8 weeks on serum lipid peroxide.

Parameter	Treatments	G1	G2	G3	G4
	Statistics	(-)ve Control	(+)ve Control	Fenugreek leaves	Fenugreek seeds
Lipid peroxidase nmol/g.tissue	Mean±SE	626.77±28.05	792.33±31.28	652.52±23.91	562.67±37.28
	T test		-4.23***	22.47***	12.95***

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant *** P<0.001.

G1: Control (-)ve: Normal rats fed on basal diet. G2: Control (+)ve: Rats treated with 2% cholesterol and fed on basal diet.

G3: Rats treated with 2% cholesterol and treated with fenugreek leaves.

G4: Rats treated with 2% cholesterol and treated with fenugreek seeds.

3.5. Renal functions

Table (5) shows the effect of either fenugreek leaves or seeds treatment on renal functions in hypercholesterolemic rats for 8 weeks. All the tested kidney function were increased as a result of hypercholesterolemia induction. As shown in Table (5), the value of uric acid of fenugreek seeds treated group that was significantly (at P<0.05) lower than that of the positive control group (1.90±0.14 and 2.70±0.46 mg/dl, respectively), whereas the mean value of uric acid in the fenugreek leaves treated group was non significantly less than that of the positive control group (1.75±0.46 and 2.70±0.46 mg/dl, respectively).

For serum creatinine, except for the mean value of fenugreek seeds treated group that was very high

significantly (at P<0.001) lower than that of the positive control group (0.64±0.01 and 0.76±0.02 mg/dl, respectively), all other mean values of creatinine were nonsignificantly less than that of the positive control group.

The fenugreek leaves treated was significantly (at P<0.05) lowered the urea value compared with that of the positive control (24.21±7.24 and 40.65±2.86 mg/dl, respectively). In addition, the fenugreek seeds treated group was also nonsignificantly lowered the urea value compared with that of the positive control (28.48±8.72 and 40.65±2.86 mg/dl, respectively).

Table (5): Effect of treating hypercholesterolemic rats with fenugreek leaves or seeds for 8 weeks on renal functions

Parameters	Treatments	G1	G2	G3	G4
	Statistics	(-)ve Control	(+)ve Control	Fenugreek leaves	Fenugreek seeds
Uric acid mg/dl	Mean±SE	2.63±0.11	2.70±0.46	1.75±0.46	1.90±0.14
	T test		-0.15 ^{NS}	1.65 ^{NS}	2.18*
Creatinine mg/dl	Mean±SE	0.74±0.02	0.76±0.02	0.74±0.00	0.64±0.01
	T test		-0.60 ^{NS}	0.92 ^{NS}	5.75***
Urea mg/dl	Mean±SE	31.86±5.174	40.65±2.86	24.21±7.24	28.48±8.72
	T test		-1.625 ^{NS}	2.23*	1.58 ^{NS}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant, *P<0.05, *** P<0.001.

G1: Control -ve: Normal rats fed on basal diet. G2: Control +ve: Rats treated with 2% cholesterol and fed on basal diet.

G3: Rats treated with 2% cholesterol and treated with fenugreek leaves.

G4: Rats treated with 2% cholesterol and treated with fenugreek seeds.

3.6. Serum electrolytes

Table (6) shows the effect of fenugreek leaves or seeds on serum electrolytes in hypercholesterolemic rats for 8 weeks. As shown the mean value of serum calcium of negative control group was significantly (at P<0.05) higher than that of the positive control (9.83±0.21 and 8.61±0.37 mg/dl, respectively), the mean calcium value in the fenugreek leaves treated group was very high significantly (at P<0.001) lower than that of the positive control (6.48±0.18 and 8.61±0.37 mg/dl, respectively). Similarly, the mean calcium value of fenugreek seeds treated group was very high significantly (at P<0.001) higher than that of the positive control group (12.90±0.20 and 8.61±0.37 mg/dl, respectively). As shown in the same Table, the value of serum sodium of fenugreek seeds treated group was high significantly (at P<0.01) higher than

that of the positive control group (190.68±14.55 and 156.78±6.10 mmol/l, respectively) and the mean value of sodium in the fenugreek treated group was nonsignificantly higher than that of the positive control group (161.8±16.45 and 156.78±6.10 mmol/l, respectively). The mean value of serum phosphorus of fenugreek leaves treated group was significantly (at P<0.05) higher than that of the positive control group (6.58±0.36 and 4.98±0.67 mg/dl, respectively), whereas the mean value of fenugreek seeds treated group was high significantly (at P<0.01) higher than that of the positive control (7.61±0.58 and 4.98±0.67 mg/dl, respectively). Serum potassium of fenugreek leaves and seeds treated groups were nonsignificantly higher than that of the positive control group (8.80±1.92, 14.11±8.56 and 5.01±0.27 mmol/l, respectively).

Table (6): Effect of treating hypercholesterolemic rats with either fenugreek leaves or seeds for 8 weeks on serum electrolytes; calcium, sodium, phosphorus and potassium.

Parameters	Treatments	G1	G2	G3	G4
	Statistics	(-)ve Control	(+)ve Control	Fenugreek leaves	Fenugreek seeds
Calcium mg/dl	Mean±SE	9.83±0.21	8.61±0.37	6.48±0.18	12.90±0.20
	T. test		2.37*	5.04***	-9.50***
Sodium mmol/l	Mean±SE	136.53±7.93	156.78±6.10	161.8±16.45	190.68±14.55
	T. test		-1.75 ^{NS}	-0.39 ^{NS}	-2.94**
Phosphorus mg/dl	Mean±SE	4.60±0.48	4.98±0.67	6.58±0.36	7.61±0.58
	T. test		-0.47 ^{NS}	-2.20*	-3.31**
Potassium mmol/l	Mean±SE	4.78±0.46	5.01±0.27	8.80±1.92	14.11±8.56
	T. test		-0.37 ^{NS}	-1.76 ^{NS}	-1.03 ^{NS}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant *P<0.05 **P<0.01 ***P<0.001.

G1: Control (-)ve: Normal rats fed on basal diet. G2: Control (+)ve: Rats treated with 2% cholesterol and fed on basal diet.

G3: Rats treated with 2% cholesterol and treated with fenugreek leaves.

G4: Rats treated with 2% cholesterol and treated with fenugreek seeds.

3.7. Histopathology

3.7.1. Kidney

Figure (1) shows the histopathology of Kidney. The negative control group rats showing normal renal structure with regulated nuclear arrangement of uriniferous tubules and collecting tubules with glomerulus (Figure 1A). The renal tissues of hypercholesterolemic rats fed 2% cholesterol for 8

weeks showing disrupted small uriniferous tubule and dilated and shrunken glomeruli led to a dilated urinary and collecting tubular space (Figure 1B). Figure (1C) shows renal tissues of fenugreek leaves treated group for 8 weeks with nearly restored normal renal tissues. Figure (1D) shows renal tissues of fenugreek seeds treated group for 8 weeks with restored normal renal tissues.

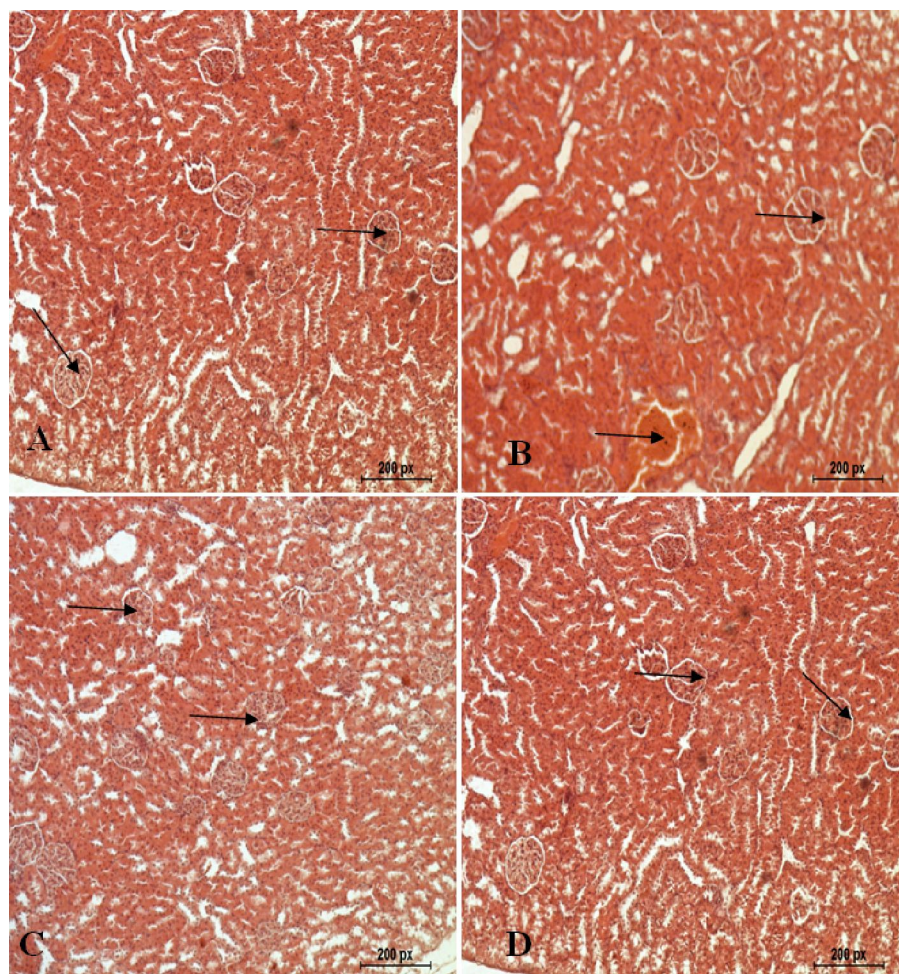


Figure (1): Histopathology of Kidney. **A;** Renal tissues of negative control group fed basal diet. **B;** Renal tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes. **C;** Renal tissues of fenugreek leaves treated group fed 2% cholesterol and co-supplemented with 500mg/kg body weight fenugreek leaves powder for 8 weeks showing restored normal configuration. **D;** Renal tissues of fenugreek seed treated group fed 2% cholesterol and co-supplemented with 500mg/kg body weight fenugreek seeds powder for 8 weeks showing restored normal configuration. Arrows: glomeruli. (H&E).

3.7.2 Liver

Figure (2) shows the histopathology of liver. The hepatic tissues of the negative control group rats show normal hepatic strands of cells and blood sinusoids Figure (2 A). Figure (2 B) shows the hepatic tissue hypercholesterolemic rats of the positive control group fed 2% cholesterol for 8 weeks with fatty liver tissue has disrupted cells with disrupted hepatic strands and vacuolated cytoplasm and necrosis. Figure (2 C) shows the hepatic tissues

of hypercholesterolemic rats of the third group treated with fenugreek leaves for 8 weeks showing restored normal appearance of the hepatic strands with well defined hepatic cords containing polyhedral hepatocytes and normal appearing round nuclei. Figure (2 D) shows hepatic tissues of hypercholesterolemic rats treated with fenugreek seeds for 8 weeks with restored normal hepatic strands.

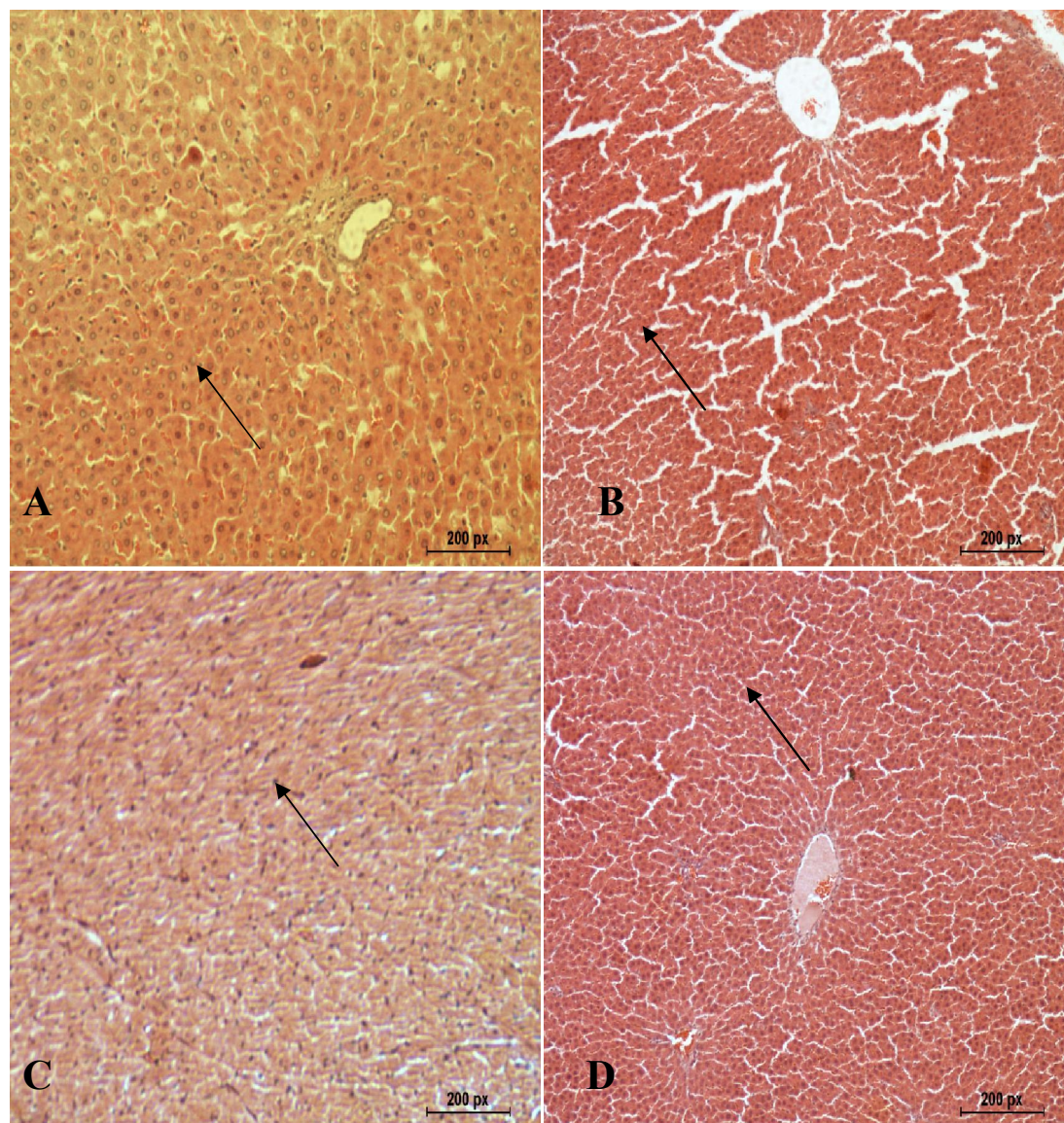


Figure (2): Histopathology of Liver. **A;** Hepatic tissues of negative control group fed basal diet (arrow). **B;** Hepatic tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes (arrow). **C;** Hepatic tissues of fenugreek leaves treated group fed 2% cholesterol and treated with 500mg/kg body weight fenugreek leaves powder for 8 weeks showing restored normal configuration (arrow). **D;** Hepatic tissues of fenugreek seed treated group fed 2% cholesterol and treated with 500mg/kg body weight fenugreek seeds powder for 8 weeks showing restored normal configuration (arrow). (H&E).

3.7.3 Testes

The testicular structure of the testis is shown in Figure (3). The testicular tissues of the negative control group rats showed normal and regular seminiferous tubules (Figure 3 A) and Figure (3B) shows the testicular structure of the testis of hypercholesterolemic group rats fed 2% cholesterol for 8 weeks suffering pathologic effects with severely damaged seminiferous tubules showing thickened

tubular walls and decreased germinal cells Figure (3C) shows testicular structure of hypercholesterolemic rats of the third group treated with fenugreek leaves for 8 weeks with restored normal structure and regular seminiferous tubules. Figure (3D) shows the testicular structure of the fourth group hypercholesterolemic rats treated with fenugreek leaves for 8 weeks with restored normal seminiferous tubules and normal germinal cell layers.

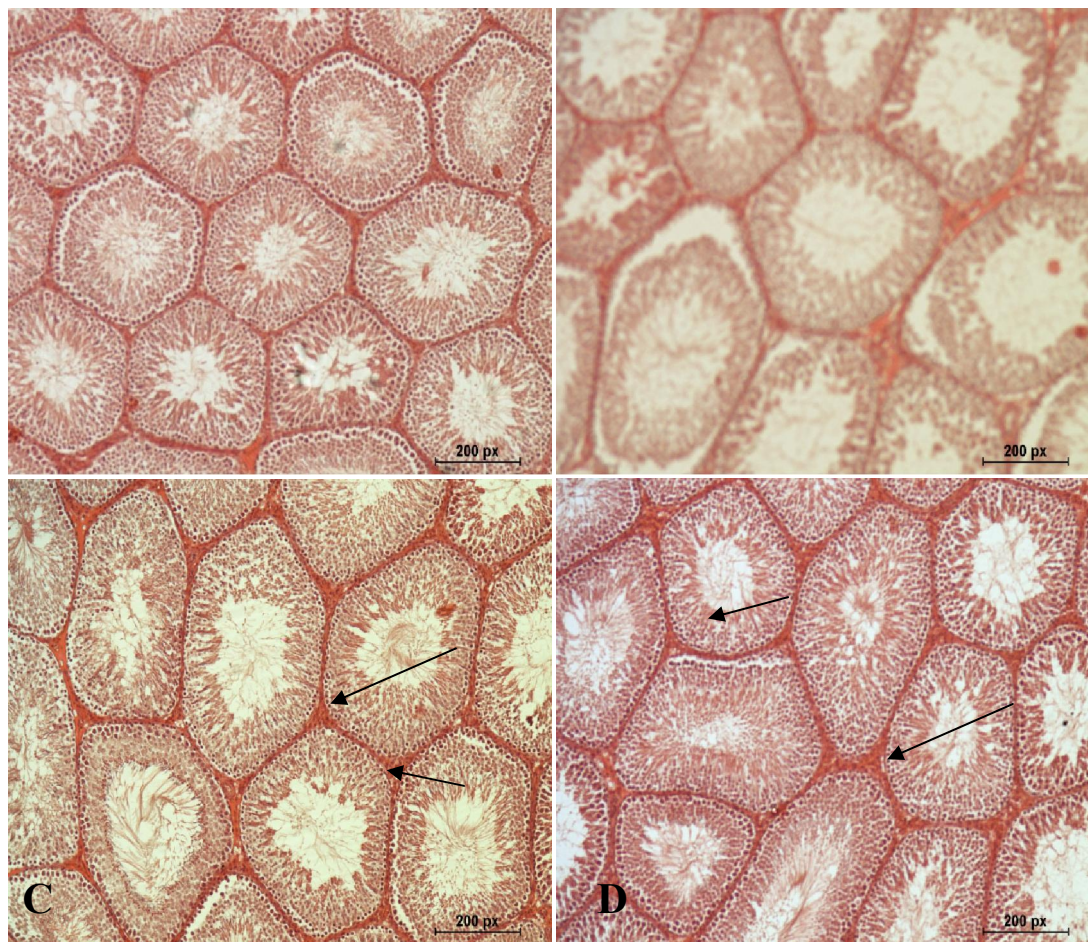


Figure (3): Histopathology of Kidney. A; Testicular tissues of negative control group fed basal diet with normal seminiferous tubules (arrow). B; Testicular tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes showing thickened tubular walls. C; Testicular tissues of fenugreek leaves treated group fed 2% cholesterol and treated with 500mg/kg body weight fenugreek leaves powder for 8 weeks showing restored normal configuration. D; Testicular tissues of fenugreek seed treated group fed 2% cholesterol and cosupplemented with 500mg/kg body weight fenugreek seeds powder for 8 weeks showing restored normal configuration. Arrows: seminiferous tubules, Tubular walls: short arrow, (H&E).

7-4 Heart

Figure 4 shows histology of heart. Cardiac tissues of the negative control group rats showing normal structure of cardiac muscles (Figure 4 A). Figure (4 B) shows cardiac tissues of hypercholesterolemic rats fed 2% cholesterol of the second group fed for 8 weeks showing increased hyalinization with cardiac muscles damage and necrosis of muscle fibers. Figure (4C) shows cardiac

tissues of hypercholesterolemic rats of the third group treated with fenugreek leaves for 8 weeks showing minimal cardiac muscles damage and nearly restored normal tissues. Figure (4 D) shows cardiac tissues of hypercholesterolemic rats of the fourth group treated with fenugreek seeds for 8 weeks showing disappearance of pathological changes and restored normal cardiac tissue structure.

4. Discussion

Hypercholesterolemia often occurs in conjunction with other metabolic risk factors including glucose intolerance, obesity, diabetes and metabolic syndromes and oxidation of the lipid core of low-density lipoproteins leads to a change in the lipoprotein conformation (Rathod et al., 2011). In the present study, feeding rats on 2% cholesterol increased the serum total cholesterol in the positive control group (Onody et al., 2003 and El Rabey et al., 2013). Treating hypercholesterolemic rats with fenugreek

leaves or fenugreek seeds (500 mg/kg body weight in the diet for 8 weeks) has significantly reduced the serum lipid profile.

Feeding male rats on 2% cholesterol has significantly increased blood lipid parameters specially, serum total cholesterol, serum triglycerides, low and very low density lipoprotein and decreased the high density lipoproteins (Onody et al., 2003, Rathod et al., 2011; Belguith-Hadriche et al., 2013 and El Rabey et al., 2013).

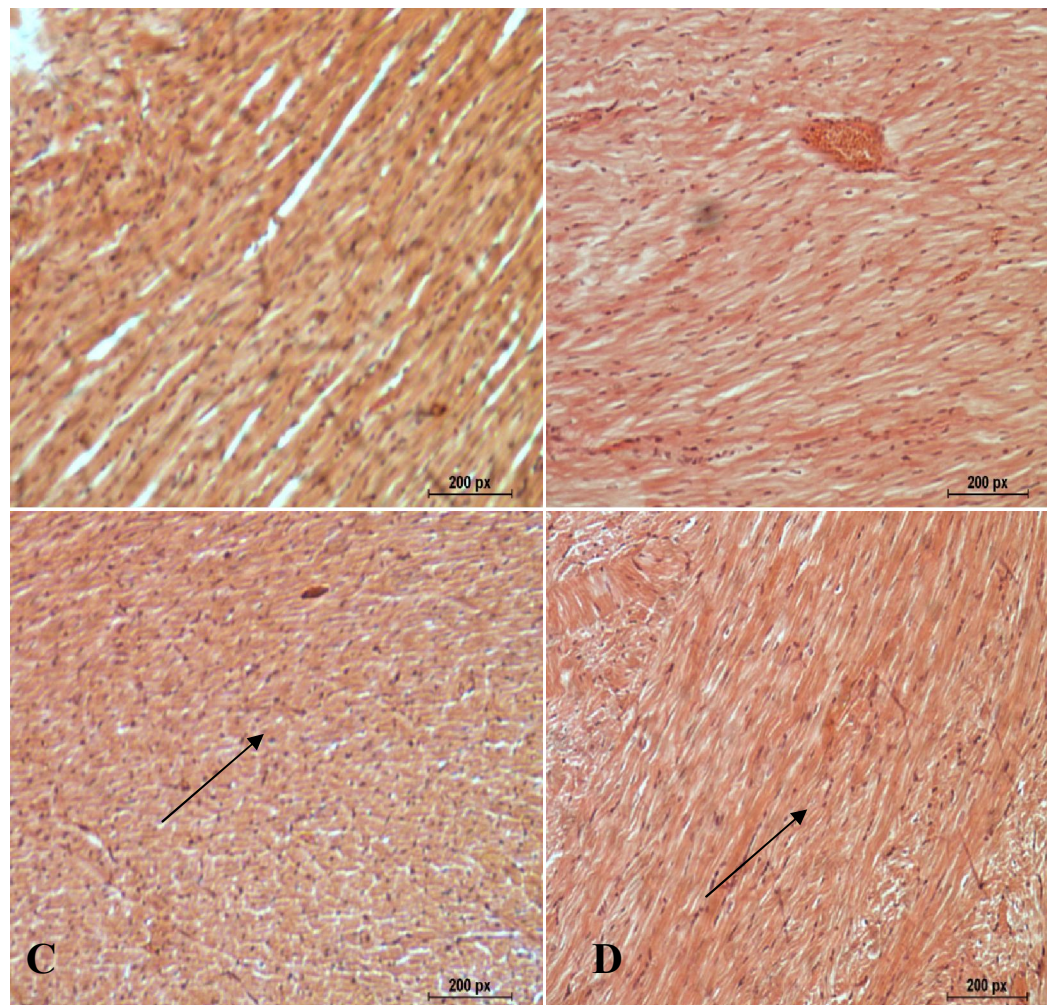


Figure (4): Histopathology of Heart. **A;** Cardiac tissues of negative control group fed basal diet. **B;** Cardiac tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes. **C;** Cardiac tissues of fenugreek leaves treated group fed 2% cholesterol and treated with 500mg/kg body weight fenugreek leaves powder for 8 weeks showing restored normal configuration. **D;** Cardiac tissues of fenugreek seed treated group fed 2% cholesterol and treated with 500mg/kg body weight fenugreek seeds powder for 8 weeks showing restored normal configuration. Arrows: muscle fibers. (H&E).

Meanwhile, treating these hypercholesterolemic male rats with fenugreek leaves or fenugreek seeds have significantly ameliorated lipid profile by lowering serum total cholesterol, serum triglycerides,

low and very low density lipoprotein and increasing the high density lipoproteins. This result is consistent with that of Bentley et al. (2002) and Makni et al. (2008).

Induced hypercholesterolemic as a result of feeding male rats on 2% cholesterol has significantly increased liver enzymes activity (S.ALT, S. γ -GT and S.ALP), whereas treating the hypercholesterolemic male rats with fenugreek leaves or fenugreek seeds have significantly ameliorated the liver enzymes under study (Mahfouz and Kummerow, 2000 and El Rabey et al., 2013).

The antioxidant enzymes (catalase, SOD and Glutathione reductase) were significantly decreased as a result of induced hyperlipidemia, whereas lipid peroxide was increased.

Treating these rats with fenugreek leaves or seeds has significantly ameliorated these parameters by increasing the levels of antioxidant enzymes and reducing the lipid peroxide (Choudhary et al., 2001; Kaviarasan et al., 2007 and Belguith-Hadriche et al., 2013).

Renal parameters were also affected by hyperlipidemia as revealed by the increased levels of creatinine, uric acid and urea (El Rabey et al., 2013). Treating these rats with fenugreek leaves or seeds has significantly ameliorated these parameters by lowering these renal parameters in the treated groups. This result is consistent with previous investigations (Ghule et al., 2012; Sindhu et al., 2012 and Kenny et al., 2013).

In addition, the present study showed disturbed serum electrolytes (calcium, sodium, phosphorus and potassium) as a result of hypercholesterolemia. This might be due to affecting glomerular filtration rate or disorders in membrane permeability in the kidney (Ganong, 1999 and El-Missiry et al., 2001). Treating these rats with fenugreek leaves or seeds has restored the electrolytes to the normal levels in the treated groups. This result is consistent with previous investigations (Sindhu et al., 2012 and Kenny et al., 2013).

The histopathological investigations, showed histological alteration in the target organs (kidney, liver, testes and heart) in the 2% cholesterol fed group (G2). This result supported with previous studies suggesting a correlation between hypercholesterolemia and histological changes in the organs (Altunkaynak et al., 2008; Dimitrova-Shumcovska et al., 2010, Ouvrier et al., 2010 and El Rabey et al., 2013). An improvement in microscopic examination of tissues in groups fed on either fenugreek leaves or fenugreek seeds showed a protective role against these histopathological alterations and restored the normal histology of the target organs due to their higher content of antioxidant that help to prevent cell damage that work in association with enzymes and reduces the effect of dietary cholesterol resulting in restoring the normal histology of the target organs (Chatterjee and

Sharma, 2010; Liu et al., 2012 and El Rabey et al., 2013).

It could be concluded that treating the hypercholesterolemic rats with fenugreek leaves or seeds has improved the biochemical blood tests and the histology of the studied organs tissues. This is might be to the fact that fenugreek leaves and fenugreek seeds have an anti-oxidant activity that ameliorated the hyperlipidemia, improved liver and kidney functions and decreased lipid peroxide in hypercholesterolemic male rats. The antihyperlipidemic activity of fenugreek could be attributed to inhibiting oxidative stress.

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