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Efficacy of lycopene on modulation of renal antioxidant enzymes, ACE and ACE gene expression in hyperlipidaemic rats

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Abstract

Introduction: We aimed to evaluate the efficacy of lycopene on renal tissue antioxidant enzymes and angiotensin converting enzyme (ACE) gene expression and serum activity in diet-induced hyperlipidaemia.

Methods: Thirty-two female Wistar albino rats (200–250 g weight), 5–6 months of age, were randomly selected and divided into four groups. Group I received normal diet; group II received 24 g high fat diet/100 g of daily diet; group III received 24 g high fat diet/100 g daily diet and 200 ml of lycopene extract (twice a week) for 8 weeks; and group IV received 200 ml oral lycopene extract twice a week for 8 weeks.

Results: A marked increase was observed in plasma urea and creatinine levels, serum C-reactive protein, kidney weight, tissue renal malonyldialdehyde level, ACE gene expression and serum level, while a decrease catalase level among hyperlipidaemic rats was observed. Histologically, interstitial inflammation and proliferation was seen. Lycopene supplementation significantly decreased plasma urea and creatinine, serum ACE, renal tissue malonyldialdehyde level and C-reactive protein level, while it increased tissue antioxidant enzymes level and total protein. Tissue inflammation and proliferation was improved.

Conclusions: This finding suggests that supplementation of lycopene is effective for renal antioxidant enzymes, ACE gene expression and ACE serum level in hyperlipidaemic rats.

Keywords

Lycopene, hyperlipidaemia, angiotensin converting enzyme

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Introduction

Lipid imbalance has long been considered as the causative factor for the development of coronary artery disease (CAD).[1] Lipid deposition in the vascular wall, the fundamental mechanism of CAD starts from infancy and progresses with age.[2] Atherosclerosis is the disorder of hyperlipidaemia, increased intracellular generation of lipid peroxidation, vascular intima media lipid deposition and cytokine imbalance.[3] Atherosclerosis is considered as an underlying phenomenon of CAD. Age, sex, genetic predisposition are non-modifiable risk factors for CAD, while diabetes mellitus, hypertension, obesity, sedentary lifestyle, smoking and dyslipidaemia are modifiable risk factors.[4]

Angiotensin II is a biologically active product of the renin–angiotensin–aldosterone system (RAAS);[5] it is a potent vasoconstrictor and regulates cerebral blood flow. The RAAS has been recognised as the principal therapeutic target for the treatment of cardiovascular diseases.[6] Ferrario in 2006[7] found that kinase II peptide has an identical structure to the angiotensin converting enzyme (ACE) inhibitor. Kinase II peptide was found to degrade bradykinin, lower blood pressure in hypertensive patients and

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beneficially modulate haemodynamics in patients with heart failure. Since then many ACE inhibitors have been studied for antihypertensive/cardiomodulatory therapies.[7]

Pharmacological therapies have been used to treat hyperlipidaemia but exert side effects. Therefore, the choice of non-pharmacological therapies including diet plays an important role in alleviating the major cause of death all over the world including cardiac heart disease, stroke, cancer, diabetes mellitus and atherosclerosis.[8] Plants have a long history of medicinal usage to treat body ailments. Antioxidants, flavonoids, polyphenols of food have been studied to treat life-threatening diseases. Lycopene is a most powerful antioxidant, which is naturally present in carrots, tomatoes, watermelons, papaya, cherries, strawberries, beans etc.[9] Previous investigations reflect the cardioprotective,[10] antineoplastic[11] and nephroprotective effects of lycopene in diabetes.[12] Therefore, the purpose of the present study is to investigate the role of lycopene on the modulation of renal antioxidant enzymes, serum ACE and ACE gene expression in diet-induced hyperlipidaemia using an experimental rat model.

Materials and methods

Animals and diet

Thirty-two female albino Wistar rats (200–260 g bodyweight) were purchased from the animal house of the International Center for Chemical and Biological Sciences, Karachi, Pakistan, for the study. Animals were kept in the laboratory for one week before the start of the experiment and were caged in a temperature controlled room (23±4°C). Drinking water, standard rat diet and high fat diet (HFD) for the experiment were easily available to the rats. The experiments were followed in accordance with the ethical guidelines of internationally accepted principles for laboratory use and care in animal research (Health Research Extension Act of 1985).[13]

Preparation of HFD

A HFD was prepared according to the method of Khalifa et al.,[14] 30 g of casein, 7 g wheat flour, 4 g bran, 10 g glucose, 6 g common salt, 3% vitamin mixture and 40 g raw melted beef fat separately. All ingredients were then mixed with water and 12 g pellets were prepared. Almost 50.4% of the daily ingested calories through this diet were derived from its fat content.

Preparation of lycopene extract

The lycopene extract was prepared according to the method of Lavecchia and Zuorro.[15] Fresh tomatoes were washed with tap water and an X cut was made at the small end, they were then immersed in boiling water for about 1–2 minutes to remove the skin, and dried in an oven at 200°C to remove moisture. About 250 mg of dehydrated peel was mixed with 3 ml of butylated hydroxytoluene in acetone (0.5% w/v), 3 ml of ethanol, 6 ml of hexane and incubated in a 25°C water bath with continuous stirring. After 2 hours, 2.5 ml of aqueous enzyme solution (pectinase 2% v/v in glycine buffer pH 10) was added and the mixture was then left for 20 hours; 7.5 ml of hexane was then added with continuous stirring and allowed to stand for a further 4 hours, so that the organic and aqueous phase was separated and the upper most reddish coloured hexane layer was extracted as lycopene. Spectrophotometrically the lycopene concentration was determined as 503 nm with absorbent coefficient 3120 for hexane as blank. The lycopene concentration obtained per litre was 0.437 mg.

Study design

Thirty-two albino wistar rats of female sex (200–250 g bodyweight) were randomly divided into four groups. Each group consisted of eight rats (n=8).

Group I: Control group (negative controls) received normal rat diet.

Group II: Hyperlipidaemic positive controls received 24 g of HFD/100 g of daily diet for 8 weeks. Animals were weighed before HFD and then every 7 days.

Group III: Hyperlipidaemic plus lycopene-treated group received 24 g HFD/100 g of daily diet plus 200 ml oral lycopene extract (twice a week) for 8 weeks.

Group IV: Lycopene-treated group received 200 ml of lycopene extract (≈87.4 μg of lycopene) orally twice a week for 8 weeks.

Sample collection

After 12 hours of fasting, the rats were dissected under anaesthesia. A 5 ml heparin-coated syringe with 25–30 gauge size needle was inserted from behind the xiphoid cartilage into the heart, making an angle of 10–30°C from the breast bone. About 10–20 ml of blood was collected through this procedure. The plasma was obtained by collecting blood in heparin-coated glass tubes and centrifuging at 3000×$\boldsymbol{g}$ for 5 minutes and storing at −80°C for biochemical analysis.

The serum was obtained by collecting the blood in clean glass tubes and allowing it to stand for 1–2 hours undisturbed. It was then centrifuged at 3000 rmp for 5 minutes and stored supernatant at −80°C for biochemical analysis.

After dissection, the kidneys were removed from the animals, washed with ice cold saline (0.9% NaCl), adherent fat and connective tissues were removed and kidneys were blot dried over the filter paper. Both kidneys were

weighed and one was stored at −80°C for preparing homogenate, while the other kidney was used for histopathological study. The kidney was immersed in 10% formalin, dehydrated and embedded in paraffin, sectioned at 3 μm, stained with haematoxylin and eosin and evaluated by light microscopy.[16]

For homogenate preparation: Kidney tissues at a ratio of 1:10 (w/v) were mixed with 100 mM potassium chloride buffer of pH 7.0 and homogenised, then centrifuged at 600×***g*** for 60 minutes at 4°C. Supernatant was separated and stored at −70°C for analysis of antioxidant enzymes;[17] 10 μl of butylated hydroxytoluene (0.5 M in acetonitrile) was added to prevent homogenate from oxidation and the homogenate was stored at −70°C until analysis for malonyldialdehyde.

Biochemical analysis

For the assessment of kidney function plasma samples were assayed for urea[18] and creatinine.[19] Urea was estimated by an enzymatic method and creatinine by Jaffe's method.

Plasma total protein was analysed by the Biuret method.[20] Serum C-reactive protein was estimated by particle enhanced turbidimetric immunoassay using the Biolatex kit,[21] nitrite by Griess' reaction.[22] For electrolyte analysis the end point method was used. Serum sodium and potassium were analysed by the method of Henry et al.,[23] serum calcium by the method of Moorehead and Biggs,[24] and serum chloride by the method of Feldkamp et al.[25]

For serum ACE activity the colorimetric enzymatic assay by Studdy and Bird[26] was used.

Analysis of ACE gene expression: ACE gene expression was estimated by the method of Korstanje et al.[27] A centimetre of tissue was treated with 500 μl of digestion buffer (1 mg/ml of proteinase K, 50 mM Tris-Cl of pH 8.0, 100 mM NaCl, 100 mM ethylenediamine tetraacetic acid (EDTA) of pH 8.0 and 1% sodium dodecyl sulphate (SDS)) at 55°C. Phenol, chloroform and isoamyl alcohol was added to the mixture at a ratio of 25:24:1, centrifuged at 14,000 rpm for 5 minutes at room temperature then allowed to precipitate by adding 100% ethanol (2 volumes). A dried pellet of DNA was suspended in 1 ml of Tris EDTA buffer of pH 7.5–8.0. Quantitative reverse transcriptase polymerase chain reaction was used to assess ACE gene expression.

Analysis of renal tissue oxidant status

Tissue catalase was estimated by the method of Sinha.[28] Briefly, tissue homogenate was mixed with phosphate buffer of pH 7.0 and added to 1 ml of 0.2 M hydrogen peroxide solution. Then it was added to 2 ml of 5% dichromate acetic acid solution and boiled for 10 minutes, cooled with tap water and absorbance was read at 570 nm on a Schimadzu spectrophotometer UV-120-01. The activity was calculated as μm/g of tissue.

Tissue superoxide dismutase (SOD) was estimated by the method of Kono.[29] Briefly, the reaction mixture consisted of 1.3 ml of EDTA–Na_2CO_3 solution, 0.5 ml of 90 μm nitroblue tetrazolium solution (NBT), 0.1 ml of Triton X-100 EDTA solution of pH 10.0 and 0.1 ml 20 mM hydroxylamine hydrochloride solution of pH 6.0. The rate of NBT reduction of the reaction mixture was recorded per minute on a Schimadzu UV spectrophotometer at 560 nm then added to 0.1 ml of tissue homogenate. The percentage inhibition of NBT reduction rate was recorded at U/g of tissue.

Tissue glutathione was estimated by the method of Carlberg and Mannervik.[30] Briefly, the reaction mixture consisted of 0.1 ml tissue homogenate, 0.3 ml of 10% bovine serum albumin, 1.5 ml 50 mM Potassium phosphate buffer (KPO_4) buffer of pH 7.6, 0.35 ml of 0.8 Mm β reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 0.1 ml of 30 mM oxidised glutathione. Absorbance was recorded on a kinetic spectrophotometer PRIM 500 for 5 minutes at 25°C. The tissue glutathione level was estimated as U/g of tissue.

Tissue malonyldialdehyde was estimated by the method of Okhawa et al.[31] Briefly, the reaction mixture consisted of 0.2 ml of SDS, 1.5 ml of acetic acid of pH 3.5, 1.5 ml of aqueous thiobarbituric acid and 0.2 ml of tissue homogenate. Distilled water was added to make up the volume to 4 ml, the mixture was then heated at 95°C for 60 minutes, cooled with tap water and centrifuged. Then 1 ml of distilled water and 5 ml of n-butanol and pyridine (15:1 v/v) were added. A pink coloured organic layer was read at 532 nm on a Schimadzu UV spectrophotometer. The tissue malonyldialdehyde concentration was measured as nmol/g of tissue.

Results

Histological examination of hyperlipidaemic rats showed proliferation with moderate interstitial inflammation. While lycopene-treated rats showed no such proliferation as shown in group III; however, mild interstitial inflammation was observed. Alone lycopene-treated rats showed no significant changes when compared with the positive controls.

The urea level was significantly increased in hyperlipidaemic rats when compared with negative controls ($P<0.005$). A partial decrease was observed when treated with lycopene as shown in group III when compared with the positive controls ($P<0.05$), while lycopene treatment also showed a significant decrease in the urea level when compared with negative/positive controls. The creatinine level was significantly increased in hyperlipidaemic rats ($P<0.005$) when compared with negative controls, while no significant results were observed with lycopene treatment when compared with positive controls.

Table 1. Plasma urea and creatinine level in groups I, II, III and IV.

Parameters	Group I n=8	Group II n=8	Group III n=8	Group IV n=8	P value P<0.05
Urea (mg/dl)	33.2±0.7	52.8±1.4***	45.9±2.1[bc]	33.3±0.2[d,e/e]	a/b
Creatinine (mg/dl)	0.49±0.02	1±0.09***	0.79±0.02[ac]	0.55±0.02[e/d,e]	a/b

Statistical probability of **P<0.01, *P<0.05, ***P<0.005 was considered to be significant for calculating the difference between negative and positive controls by t-test.
ns: considered non-significant when compared with negative/positive controls.
Significant analysis of variance test is presented as a: when compared with negative controls; b: compared with positive control; c: compared controls with group III; d: compared controls with group IV, e: compared group III with group IV.

A marked increase in the CRP level was observed in hyperlipidaemic rats when compared with negative controls (P<0.005). Lycopene extract treatment showed a slight decrease in the CRP level observed in group III when compared with positive controls.

No changes were observed in total protein level in hyperlipidaemic rats when compared with negative controls. Administration of lycopene showed a significantly increased protein level when compared with positive controls (P<0.05).

No significant results were observed in the electrolyte level when compared with positive controls, while a significantly increased potassium level was observed when compared with negative controls (P<0.05).

A significant increase in the weight of both kidneys was observed in hyperlipidaemic rats (P<0.05). Lycopene treatment showed no significant changes in the weight of both kidneys when compared with negative controls, while lycopene extract treatment showed a significantly decreased weight of both kidneys (P<0.05) when compared with positive controls.

The tissue catalase level was significantly decreased in hyperlipidaemic rats (P<0.05) when compared with negative controls. Lycopene treatment showed no significant results when compared with negative/positive controls.

The tissue SOD level showed no significant change in hyperlipidaemic rats when compared with negative controls. Similarly, no significant changes were observed in the SOD level when compared with negative/positive controls.

The tissue glutathione level showed no change in hyperlipidaemic rats when compared with negative controls. Lycopene treatment showed a marked increase in the glutathione level when compared with positive controls (P<0.05).

The tissue malonyldialdehyde level was markedly increased in hyperlipidaemic rats when compared with negative controls (P<0.01). Lycopene treatment showed a markedly decreased MDA level when compared with positive controls (P<0.05).

The serum nitrite level was significantly increased in hyperlipidaemic rats (P<0.05) when compared with negative controls. Lycopene treatment showed a slightly increased nitrite level when compared with negative controls, while no changes were observed when compared with positive controls.

A marked increase in ACE gene expression (P<0.05) and serum ACE level (P<0.005) was observed in hyperlipidaemic rats when compared with negative controls, while lycopene treatment showed a significantly decreased serum ACE level when compared with positive controls.

Statistical analysis

Results are presented as mean±SE; statistical significance and differences from controls (group I) and hyperlipidaemic controls (group II). Values were evaluated by Student's t-test. Statistical probability of **P<0.01, *P<0.05, ***P<0.005 was considered to be significant. One way analysis of variance with the post hoc t-test was used to compare the level of significance between controls and test groups. The value P<0.05 was considered significant.

Discussion

The biochemical parameters that monitored the kidneys are the markers of kidney toxicity and are used for the assessment of tissue damage. The measurement of different parameters, their levels and their activities are used for the diagnosis.[32] Hypercholesterolaemia accelerates the progression of kidney disease.[33] Urea and creatinine levels are used as an indicator of nephrotoxicity as low clearance of these substances indicates diminished or impaired ability of the kidney to clear the body's waste products.[34] We found increased urea and creatinine levels in hyperlipidaemic rats showing their nephrotoxic effect (Table 1, P<0.005). Treatment of hyperlipidaemic rats with lycopene produced significantly decreased urea and creatinine levels (P<0.05) suggesting its nephroprotective effect against hyperlipidaemia. Kidney tissues indicated from the histological examination that lycopene antioxidant activity protects the renal parenchyma from oxidative damage and thus modulates the endogenous cholesterol synthesis and metabolism and reduces the intensity of hyperlipidaemia-based nephrotoxicity (Figure 1).

Table 2. Serum CRP and plasma total protein level in groups I, II, III and IV.

Parameters	Group I *n*=8	Group II *n*=8	Group III *n*=8	Group IV *n*=8	*P*<0.05
Serum CRP (mg/dl)	9.95±0.32	15.3±0.60***	12.4±0.91$^{ns/c}$	9.7±0.1$^{d,e/d}$	a/b
Plasma total protein (mg/dl)	6.13±0.19	5.69±0.03^{ns}	6.04±0.2$^{ns/c}$	5.86±0.18$^{ns/d}$	ns/b

CRP: C-reactive protein.
Statistical probability of **P<0.01, *P<0.05, ***P<0.005 was considered to be significant for calculating the difference between negative and positive controls by *t*-test.
ns: considered non-significant when compared with negative/positive controls.
Significant analysis of variance test is presented as a: when compared with negative controls; b: compared with positive control; c: compared controls with group III; d: compared controls with group IV, e: compared group III with group IV.

Table 3. Serum electrolytes in groups I, II, III and IV.

Parameters	Group I *n*=8	Group II *n*=8	Group III *n*=8	Group IV *n*=8	*P*<0.05
Sodium (mEq/L)	142.3±1.45	142±1.0ns	144.5±1.5	147±1.0	ns/ns
Potassium (mEq/L)	3.4±0.13	5.35±0.7 ns	4.25±0.25$^{c/ns}$	4.5±0.25	a/ns
Calcium (mEq/L)	10.21±0.1	10.58±0.2 ns	9.78±1.0	9.55±0.81	ns/ns
Chloride (mEq/L)	108±2.0	109±3.0 ns	107.5±2.5	108.5±1.5	ns/ns

Statistical probability of **P<0.01, *P<0.05, ***P<0.005 was considered to be significant for calculating the difference between negative and positive controls by *t*-test.
ns: considered non-significant when compared with negative/positive controls.
Significant analysis of variance test is presented as a: when compared with negative controls; b: compared with positive control; c: compared controls with group III; d: compared controls with group IV, e: compared group III with group IV.

Table 4. Kidney tissue oxidative status in groups I, II, III and IV.

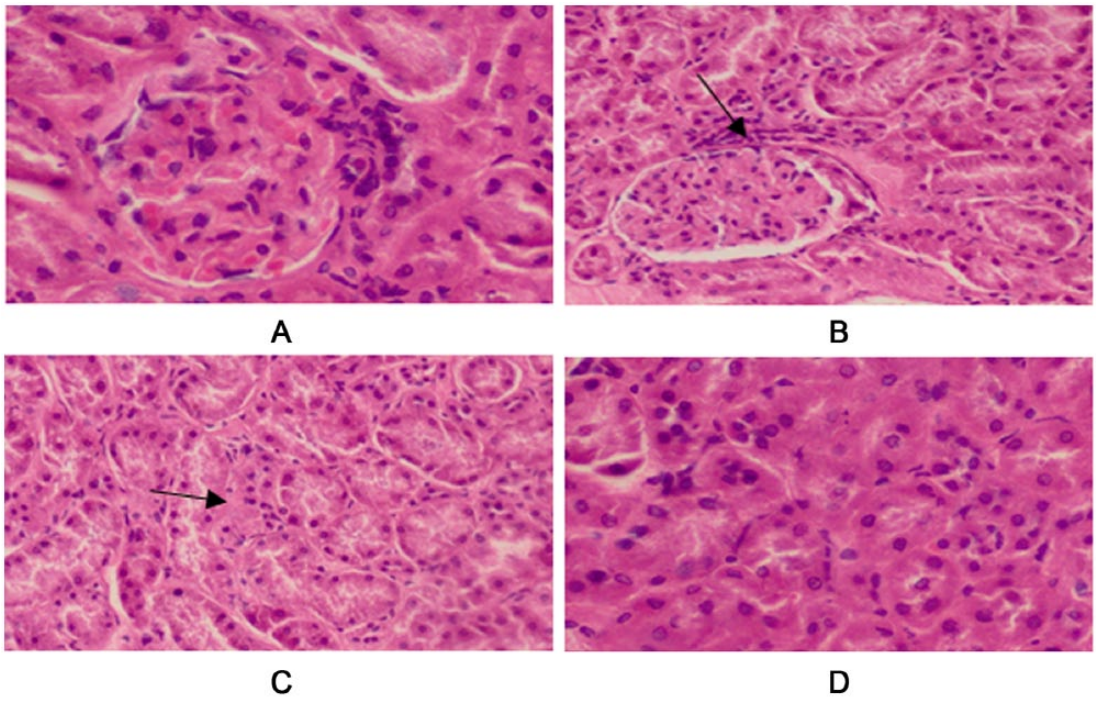
Parameters	Group I *n*=8	Group II *n*=8	Group III *n*=8	Group IV *n*=8	*P*<0.05
Right kidney weight (g)	0.45±0.03	0.61±0.05*	0.5±0.03	0.44±0.01	ns/b
Left kidney weight (g)	0.46±0.0.03	0.63±0.05*	0.50±0.03	0.43±0.01	ns/b
Catalase (µmol/g tissue)	4.79±0.22	3.9±0.22*	5.6±0.3	8.2±3.6	ns/ns
SOD (U/ml)	14.10±1.0	12.05±0.86^{ns}	15.9±1.3	14.6±0.7	ns/ns
GSH (U/g tissue)	5.4±0.65	4.25±0.78^{ns}	10.5±0.7$^{ns/c}$	10.8±0.2$^{ns/d}$	a/b
MDA (nmol/g tissue)	58.7±0.02	94.7±7.0*	86.3±9.1$^{ns/c}$	55.6±4.3$^{ns/e}$	a/b
Nitrite (µmol/L)	10.61±1.39	5.63±0.3*	6.75±0.2$^{c/ns}$	10.14±0.31$^{d/ns}$	a/ns

SOD: superoxide dismutase; GSH: glutathione; MDA: malonyldialdehyde.
Statistical probability of **P<0.01, *P<0.05, ***P<0.005 was considered to be significant for calculating the difference between negative and positive controls by *t*-test.
ns: considered non-significant when compared with negative/positive controls.
Significant analysis of variance test is presented as a: when compared with negative controls; b: compared with positive control; c: compared controls with group III; d: compared controls with group IV, e: compared group III with group IV.

Table 5. Serum ACE activity and kidney ACE gene expression changes in groups I, II, III and IV.

Parameters	Group I *n*=8	Group II *n*=8	Group III *n*=8	Group IV *n*=8	*P*<0.05
Serum ACE (U/L)	41.13±0.46	56.74±1.6***	51.46±2.12ns/c	42.9±0.99$^{ns/e}$	a/b
Tissue gene expression (U/L)	0.78±0.05	2.68±0.35*	2.4±0.20$^{ns/ns}$	0.96±0.38ns/e	a/b

ACE: angiotensin converting enzyme.
Statistical probability of **P<0.01, *P<0.05, ***P<0.005 was considered to be significant for calculating the difference between negative and positive controls by *t*-test.
ns: considered non-significant when compared with negative/positive controls.
Significant analysis of variance test is presented as a: when compared with negative controls; b: compared with positive control; c: compared controls with group III; d: compared controls with group IV, e: compared group III with group IV.



A Normal kidney histology
B Histopathological changes in kidney sections from animals of Hyperlipidemic group. Presence of mesangial matrix and interstitial inflammation
C Kidney sections from animals of Hyperlipidemia+Lycopene group showing less severe changes than Hyperlipidemic group.
D Preserved tissue histology with lycopene supplementation in animals of Lycopene treated group.

Organ appearance & Scoring of kidney tissues According to histopathology :

	Group I	GroupII	Group III	Group IV
Colour	-	Brown	Brown	brown
Texture		Smooth	Smooth	Smooth
Enlargement mesangial matrix		-	-	-
Interstitial inflammation		+2	+1	-
Fibrosis		-	-	-
Proliferation		+2	-	-

Scale: +1------- mild;+2----------moderate;+3---------- moderate to severe
+4---------- severe

Figure 1. Histopathological examination of kidney tissue in groups I, II, III and IV.

Among the numerous markers for the detection of hyperlipidaemia, an imbalance in antioxidant status was observed in diet-induced hyperlipidaemia. Increases in the activity of lipid peroxidation and oxidant enzymes included NADPH oxidase, myeloperoxidase and xanthine oxidase. NADPH oxidase has been recognised within the vascular wall and has a great impact on reactive oxygen species (ROS)-related decreased nitric oxide (NO) bioavailability.[35] In the pathological condition overactivity of these enzymes leads to the overproduction of free radicals, leading to scavenging of the NO molecule, uncoupling endothelial NO synthase or/and formation of peroxynitrite.[36] Uncoupling of endothelial NO synthase with substrate reduction can transform into a superoxide generating enzyme, which in turn increases the production of oxidant radicals, hydrogen peroxide along with NO

production.[37] A range of antioxidant mechanisms balances the effects of ROS including catalase, superoxide dismutase and glutathione. The present investigation indicated a high concentration of malonyldialdehyde ($P<0.01$) with a low tissue catalase level ($P<0.05$) (Table 4). This result is corroborated with previous investigators who found a significantly increased tissue malonyldialdehyde level in hyperlipidaemia, which was inversely proportional to antioxidant enzymes.[38] Lycopene has been shown to be one of the best biological suppressants of free radicals, especially those derived from oxygen. It has the highest singlet-quenching rate among all carotenoids in the biological system.[12] The findings in the present study demonstrated the ability of lycopene to protect the kidney tissue from cellular damage through elevation of antioxidant enzymes.

CRP is a marker of inflammation to predict cardiovascular disease among healthy humans. It is also used clinically for the assessment of low-grade cardiovascular inflammation.[39] We found a marked increased CRP level in hyperlipidaemic rats (Table 2, $P<0.005$) and found a significant effect of lycopene on hyperlipidaemic rats. CRP promotes endothelial dysfunction and is responsible for atherosclerosis.[39] Rein et al. studied tomato flavanoids and showed these substances to reduce the CRP level and fibrinogen in contrast to increased vitamin E, selenium and serum high-density lipoprotein.[40] Pasceri et al. hypothesised that CRP via interleukin 6 stimulates vascular dysfunction by inhibiting endothelial NO synthase, increases oxidative stress, vascular permeability, stimulates the adhesion molecules, chemokine production, and thrombus formation within the endothelial cells.[41] No significant changes were observed in the total protein level in hyperlipidaemic rats as shown in Table 2. While lycopene treatment significantly increased the total protein level, Ozturk et al.[42] found in their study that intoxication of carbon tetrachloride damaged the cell membrane and their intracellular organelles and increased the permeability of the basement membrane of the kidney thus increasing protein loss in the urine. Lipoprotein cholesterol is an important constituent of the plasma membrane thus a big change may increase the membrane permeability and cause lipid peroxidation.[43]

The significantly increased total protein level in group III may suggest that preferential deposition of lycopene in organs such as the liver may be implicated.[44]

Hyperlipidaemia is characterised by hyponatraemia usually giving rise to derangements of homeostatic balance with respect to electrolytes.[45] Although no changes were observed in serum electrolytes, including sodium, potassium chloride on hyperlipidaemic-treated rats when compared with negative controls, following treatment with lycopene the serum level of sodium, calcium chloride did not differ with hyperlipidaemic rats when compared. Whereas the serum potassium level showed significant results following treatment with lycopene when compared with negative controls.

Recent evidence indicates that major cardiovascular risk factors influence the renin–angiotensin system function, including an increased synthesis of the angiotensin I converting enzyme.[46] The present investigation indicates the increased ACE serum activity and gene expression in hyperlipidaemic rats. Niemiec et al. investigated hyperlipidaemia, which is one of the major risk factors of cardiovascular arterial disease, increasing the plasma concentration of angiotensinogen and the angiotensin peptides and upregulating the expression of the angiotensin II type 1 receptor (AT1R) gene.[47] Suzuki et al. found strong correlation between the DD genotype and the D allele with hyperlipidaemia.[48] In smokers, the D allele has been associated with endothelial dysfunction, resulting in increased levels of angiotensin II, which has been shown to increase the formation of SOD anions and the degradation of NO causing endothelial dysfunction.[49] Treatment of hyperlipidaemic rats with lycopene significantly reduced the serum ACE level and gene expression, suggesting that lycopene has strong anion quenching ability as compared to other carotenoids and gives a protective effect.[50]

Conclusion

The results obtained from the present study demonstrated that lycopene significantly attenuated the urea and creatinine level and also improved the tissue kidney oxidant status. Lycopene treatment also regulated the serum ACE and gene expression. Thus our findings suggest the ability of lycopene to protect the kidney tissues dysregulated by HFD-induced hyperlipidaemia.

Declaration of conflicting interest

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