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Original Article

Lutein attenuates diabetic-induced renal damage via inhibiting oxidative and nitrosative stresses

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Summary. Diabetic nephropathy is a complex disease that involves production of free radicals, which are strong stimulus for pro-inflammatory factors. Present study was designed to explore the potential alleviating effects of lutein against streptozotocin-induced diabetic renal oxidation, inflammation and apoptosis in rats. Lutein was supplemented with diets content in three different doses (40, 80 and 160 mg/kg diets). Levels of glucose, albumin, creatinine and urea were determined in serum and urine. Renal expression of pro-inflammatory cytokines, nucleic acids and caspase-3 activity were estimated and also the kidney levels of thiobarbituric acid reactive substances (TBARS), total and non-protein sulfhydryl groups (T-SH and NP-SH), nitric oxide (NO) and inducible nitric oxide synthase (iNOS) along with superoxide dismutase (SOD) and catalase (CAT) activities. Renal histopathological features were analyzed. The diabetic animals showed apparent alterations in serum and urine biochemistry and also demonstrated evident elevated renal levels of pro-inflammatory mediators and apoptosis. Measured oxidative/nitrosative stress biomarkers were found altered markedly in the renal tissues of diabetic rats. Lutein supplementation to the diabetic animals significantly improved serum and urinary biochemistry. In renal tissue, the altered pro-inflammatory cytokines, nucleic acids and caspase-3 activity were ameliorated following lutein supplementation. Diabetic animals supplemented with lutein exhibited normal values of TBARS, T-SH, NP-SH and iNOS as well as SOD and CAT activities particularly in higher dose. Histological analysis further confirmed lutein's renal protective effects. These results demonstrated clear evidence that lutein offered a significant protective effect against diabetes-induced nephrotoxicity by inhibiting the oxidative and nitrosative process in renal tissue.

Key words: lutein, nephropathy, oxidative/nitrosative stress, renal inflammation

Introduction

Diabetes mellitus (DM) is considered one of the most prevalent metabolic disorders. It has an extremely high global prevalence, which is assumed to reach 5.4% by 2025 (1). Diabetic patients have significant hyperglycemia and alteration in carbohydrate, lipid and protein metabolisms. DM is characterized by several micro and macro-complications including atherosclerosis, neuropathy, retinopathy, and nephropathy (2). Diabetes-induced nephropathy and renal inflammation are important pathological complications in both types of diabetes. They are the most prevalent causes of diabetic associated end-stage renal disease as a result of discrepancies in metabolic and hemodynamic factors (3). Diabetes-induced nephropathy is characterized by definite pathological changes including alterations in glomerular basement membrane and clinically significant glycosuria and albuminuria (4).

Hyperglycemia can provoke production of free radicals such as reactive oxygen and nitrogen species (ROS/RNS), resulting in oxidative and nitrosative stress that disrupt glomerular functions and alter cellular components (5). Elevated level of free radical generation

exerts also cytotoxic effects on the membrane phospholipids. Numerous clinical and experimental studies reported elevation of free radicals in both types of DM, where ROS and RNS depleted endogenous cellular antioxidants such as glutathione and inhibited the antioxidants scavenging enzymes activities including catalase (CAT) and superoxide dismutase (SOD) (2). Oxidative and nitrosative stress are deemed to provoke renal inflammation and apoptosis. Renal inflammation due to diabetes has marked deleterious effects on kidney function (6). In addition, oxidative/nitrosative stress associated renal inflammation are closely linked with a positive feedback loop leading to inflammatory cytokine release and ROS/RNS generation (7). Moreover, inflammation was found to trigger cultured glomerular endothelial cells apoptosis via oxidative/nitrosative stress dependent pathways (8).

Plant derived carotenoids are now considered as potent biological compounds (9). They gain a therapeutic value as they are effective against a wide range of metabolic disorders including diabetes (9). Lutein is an important member of fruits and vegetables naturally distributed carotenoids (10). Lutein is not synthetized endogenously and thus it should be obtained as dietary supplement (10). Turnip greens, dark-green leafy vegetables kale, collards and spinach are rich with lutein (11). Lutein has reported antioxidant and anti-inflammatory properties (12). Several studies demonstrated its therapeutic value, especially in different eye diseases such as age-related macular degeneration (13-15).

In this study, we investigated whether lutein via its beneficial therapeutic value as antioxidant and anti-inflammatory agent can attenuate the diabetes-induced renal damage and inflammation triggered by ROS and RNS using an experimental animal model of diabetes in Wistar rats.

Materials and methods

Animals

Male Wistar albino rats, weighing 270-290 g, were received from Experimental Animals Care Center, College of Pharmacy, King Saud University, Riyadh Saudi Arabia. They were maintained under controlled conditions of 24±1°C temperature, 12 h light/dark cycle and had free-access to water and purina rats chow. Animals' handling, treatment and anesthesia techniques were exactly used as described in the National institute of health (NIH) guideline for the care and use of laboratory animals (NIH publications no. 80-23, 1996). In addition, ethical approval number (238-EACC-2014) was obtained by the ethical committee of the Experimental Animals Care Center, College of Pharmacy, King Saud University.

Diabetes induction

Experimental diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (65 mg/kg; Sigma-Aldrich, ST. Louis, MO, USA) in 0.1 mol/l citrate buffer (pH 4.5), while control animals received equal volume of citrate buffer without STZ. Two days following STZ shoot, DM was verified by estimation of fasting blood glucose levels from animal's tail vein using Accu-chek compact-plus glucose meter system (Roche Diagnostics, Meylan, France). Animals with a glucose levels higher than 250 mg/dl were considered as diabetic.

Diet preparation

Lutein content experimental diets were prepared as pellet form by mixing the concentrations (40, 80 and 160 mg/kg diet) lutein (Carbone Scientific Co., Ltd., London, UK) with rats chow powder and shade dried. The diets were prepared freshly every week and fed for whole experimental period. All group of animals were kept on free access to food and water for 5 weeks. Lutein doses were designed based on the diabetic animals food daily intake as 35 to 40 g per day, thus the range of our doses will be in between 1.5 to 6 mg/day. In previous study, lutein showed neither short-term nor long-term toxicity at 4, 40, and 400 mg/kg body weight doses for 4 and 13 weeks, respectively (16).

Experimental design

Animals were randomly allocated in five groups (n=6), as follow; (1) Control non-diabetic (C), (2) Diabetic (D), (3) Diabetic supplemented with 40 mg/kg lutein diet (D+L40), (4) Diabetic supplemented with 80 mg/kg lutein diet (D+L80) and (5) Diabetic supplemented with 160 mg/kg lutein diet (D+L160). Animals' behavior and general health was monitored

during treatment periods and their 24 h food-intake, water-intake and body weights were recorded on same day and time of every week. At the end of the fifth week, animals were allocated at metabolic cages for 24 h for urine collection. Under the light ether anesthesia, blood samples were obtained via the cardiac puncture. Animals were then euthanized and both the kidneys were immediately dissected and stored at -80˚C for analysis.

Serum and urinary analyses

Serum and urinary levels of glucose, albumin, creatinine and urea (BUN) were estimated by the commercially available diagnostic kits (Randox Laboratories Ltd., Crumlin, County Antrim, UK).

Determination of renal inflammation

Renal inflammatory process was assessed by estimating cytokines including TNF-α, IL-1β, IL-6 and IL-10 expressions in kidney homogenates using enzyme-linked immunosorbent assay (ELISA) technique following instruction provided by the manufacturer (R&D Systems, Inc., Minneapolis, MN, USA). The values were expressed as pg/mg protein.

Determination of renal apoptosis and cytotoxicity

Renal apoptosis and cytotoxicity were assessed by measuring the activity of caspase-3/CPP32 and the levels of renal nucleic acids. Caspase-3/CPP32 activity was measured using the commercially available colorimetric kit (Biovision Inc, Milpitas, CA, USA). Briefly, the pre-chilled kidney homogenates were mixed with lysis buffer followed by addition of the kit reaction buffer and DEVD-pNA substrate. The absorbance was read at 405 nm after 2 h of incubation at 37 ˚C in darkness. Nucleic acids (DNA and RNA) levels in kidney homogenates were determined by the method described by Bregman, (17). The homogenates were suspended in 10% ice-cold TCA. After, centrifugation, pellets were extracted twice by 95% ethanol and nucleic acids were isolated in 5% TCA then one portion of the extract was mixed with diphenylamine reagent for DNA determination and the other portion was treated with orcinol reagent for RNA quantification. To determine total protein values, the modified Lowry method by Schacterle and Pollack (18) was used.

Determination of renal nitrosative stress

The NO concentrations in the renal tissues were measured by the Griess test, using the commercially available kit (R&D Systems, Inc., Minneapolis, MN, USA), while the renal iNOS expression was determined by ELISA kit provided by Biotang Inc., Waltham, MA, USA. Values were expressed as μM/mg protein for NO and pg/mg protein for iNOS.

Determination of renal oxidative stress

The lipid peroxidation (LPO) byproduct, thiobarbituric acid reactive substances (TBARS) was determined by using an assay kit (Zepto-Metrix Corporation, Buffalo, NY, USA). In brief, kidney homogenate was mixed with the kit's reaction buffer and then heated for 60 min at 95˚C. The color absorbance of the pink supernatant was measured following cooling down at room temperature and results were expressed as nmole/mg protein. Kidney levels of sulphadryl groups were estimated using the method described by Sedlak and Lindsay (19). In brief, for T-SH levels tissue homogenates were mixed with 0.2 M Tris buffer (pH 8.2), 0.01 M Ellman's reagent and 5,5'-dithiobis-(2-nitro-benzoic acid) (DTNB) and the clear supernatants was measured at 412 nm after centrifugation (1800 x g for 10 min) at ambient temperature. The levels of non protein sulphadryl groups (NP-SH) in renal tissue were estimated by adding 50% TCA solution with 200 μl homogenates and shacked intermittently for l0-15 min. After, centrifugation of the samples, the supernatant was mixed with 0.4 M Tris buffer (pH 8.9) (1: ratio) and 0.1 ml DTNB and the absorbance was measured at 412 nm within 5 min. The antioxidant enzymes activities were assayed in kidney tissues. SOD activity was determined by the method described by Kono (20). The principle of the method was that the generated superoxide anions after oxidation of hydroxylamine hydrochloride can reduce the nitrobluetetrazolium to blue formazan and the extent of this reduction can be measured at spectrophotometrically. CAT activity was estimated by Aebi (21) described method. Kidney homogenates were added to 50 mM phosphate buffer and 20 mM H_2O_2 and reduction in absorbance of the produced color was recorded using spectrophotometer (Pharmacia-LKB UVM

II spectrophotometer, GE Healthcare Life Sciences, Marlborough, MA, USA).

Histopathological investigation

At the time of sacrifice, across sections of the kidney tissues were fixed in 10% formaldehyde solution. These sections were then embedded into paraffin wax blocks and cut by a microtome (American Optical Rotary Microtome, Middleton, WI, USA) into 5 μm slices. The slices were then stained with H&E and mounted. A histopathologist detected and graded the samples microscopically in a blinded manner by a Leica DM5500-B microscope (Leica Biosystems Melbourne Pty Ltd., Melbourne, Australia). A microscopic grading of all samples was expressed as mild, moderate or severe nephropathy.

Statistical analysis

Data of the current study were expressed as mean ± standard error of mean (SEM) and statistically analyzed using one-way ANOVA followed by Student-Newman-Keuls multiple comparisons test. Results were considered statistically significant when P values were ≤ 0.05. Graph Pad prism program (version 5) was employed as analyzing software (Graph Pad Software, Inc., La Jolla, CA, USA)

Results

Significant (P<0.001) decrease was found in the body weights of diabetic rats while a significant (P<0.01) increase were seen in mean kidney weights compared to control group. Lutein supplementation for five weeks with different doses could not produce any significance difference compared untreated diabetic animals (Tab. 1).

In serum, glucose, urea (BUN) and creatinine levels were significantly (P<0.001) increased while albumin levels markedly decreased compared to control values. A significant (P<0.001) increase was found in urinary levels of glucose and albumin, in contrast levels of urea and creatinine were markedly decreased in diabetic animals. Lutein (160 mg/kg) supplementation to diabetic rats significantly attenuated the serum alterations of albumin (P<0.05), urea (P<0.01) and creatinine (P<0.01), while the 80 mg/kg dose corrected (P<0.05) creatinine and urea serum levels and the 40 mg/kg dose corrected only serum creatinine levels (P<0.05). The diabetes-induced changes in the urinary levels of albumin, urea and creatinine were significantly restored following 5 weeks of lutein supplementation with 160 mg/kg diet (P<0.05, P<0.05 and P<0.01; respectively). The 80 mg/kg diet of lutein also significantly (P<0.05) increased creatinine urine levels as compared to diabetic untreated rats (Tab. 2).

Table 1. Effect of lutein supplementation on mean body and kidney weights of diabetic animals

Treatments	Body weight (g)		Kidney weight g/100 g final body weight
	Initial	Final	
C	215±5.1	327±13.1	0.65±0.02
D	212±5	190±14.7***a	1.02±0.05*a
D+L(40)	225±6.6	198±7.1	1.10±0.2
D+L(80)	232±3.7	190±9.2	1.06±0.1
D+L(160)	223±6.1	180±7.5	1.16±0.05

*Data were expressed as Mean ± SEM (n=6) and statistical analyzed using one-way ANOVA and Student-Newman-Keuls multiple comparisons test as a post hoc test. Statistical significance was considered when *P < 0.05 and ***P < 0.001. 'a'D group Vs C group and 'b lutein treated groups Vs D group.*

The levels of TNF-α, IL-1β, and IL-6 were significantly elevated (P<0.01, P<0.05 and P<0.01; respectively), while IL-10 values found significantly lower in the kidney tissues of diabetic animals compared to control rats. The higher dose of lutein diet supplementation restored these values to approximately normal levels. The median dose of lutein (80 mg/kg) diet was able to decrease the levels of TNF-α significantly (P<0.05) in renal tissue compared to normal diet fed rats (Fig. 1).

Caspase-3 activity was significantly (P<0.05) increased in the kidneys of diabetic animals compared to controls. The lutein (80 and 160 mg/kg) diets significantly (P<0.05) inhibited the increased levels of caspase-3 compared to diabetic animals. Renal DNA

Table 2. Effect of lutein supplementation on glucose, albumin, urea and creatinine levels in serum and urine of diabetic rats

Parameters (mg/dl)	C	D	D+L(40)	D+L(80)	D+L(160)
Blood glucose	131±3.2	526±27.8***a	507±16.1	494±23.3	466±28.1
Urinary glucose	67.9±12.9	522±4.4***a	506±11.6	523±3.3	408±10.7
Serum Albumin	5.5±0.2	4.4±0.2***a	4.7±0.09	4.9±0.06*b	5.01±0.01*b
Urinary Albumin	5.03±0.34	6.3±0.2**a	5.9±0.03	5.8±0.15	5.4±0.2*b
Blood urea nitrogen (BUN)	28.9±1.6	48.4±3.1***a	48.1±3.1	38.4±2.1*b	34.2±2.2**b
Urinary urea	45.4±2.1	29.2±2.3**a	33.1±3.7	40.6±2.3*b	41.2±3.4*b
Serum creatinine	0.83±0.04	1.48±0.09***a	1.25±0.08	1.16±0.06*b	0.99±0.11**b
Urinary Creatinine	161.6±14	54.55±2***a	55.97±10	89.52±9.5	114.4±12**b

*Data were expressed as Mean ± SEM (n=6) and statistical analyzed using one-way ANOVA and Student-Newman-Keuls multiple comparisons test as a post hoc test. Statistical significance was considered when *P < 0.05, **P < 0.01 and ***P < 0.001. 'a'D group Vs C group and 'b lutein treated groups Vs D group.*

and RNA levels were significantly (P<0.01) reduced in diabetic rats compared to control group. Lutein supplementation with higher taken dose significantly (P<0.01 and P<0.05; respectively) attenuated the decreased levels of DNA and RNA in renal tissue as compared to STZ group (Fig. 2).

Nitrosative stress biomarkers including iNOS and NO were significantly (P<0.01) up-regulated in the diabetic kidneys compared to control ones. Five weeks of lutein supplementation to the diabetic animals significantly (P<0.05) inhibited the levels of iNOS renal expression and NO levels as compared to diabetic untreated rats (Fig. 3).

The levels of TBARS were significantly (P<0.05) increased in diabetic animals compared to controls and in lutein diet supplemented (80 and 160 mg/kg diet) groups found significant (P<0.05) inhibition when compared to normal diet fed diabetic rats. Other oxidative stress biomarkers such as total sulphadryl groups (T-SH) and NP-SH levels were significantly (P<0.01 and P<0.05; respectively) decreased alone with SOD and CAT activities (P<0.01 and P<0.05; respectively) in renal tissues of diabetic rats compared to the control animals. Lutein content diets especially the higher dose significantly (P<0.05) corrected the inhibited levels of T-SH and NP-SH and markedly enhanced the SOD and CAT activities as compared to diabetic animals. The median dose of lutein diet supplementation also showed significant (P<0.05) increase in levels of T-SH and CAT activity (Fig. 4).

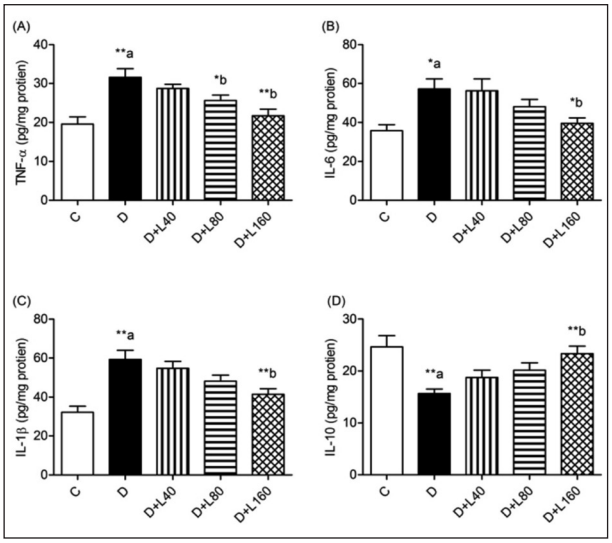

Figure 1. Effect of lutein supplementation on renal inflammatory mediators including (A) TNF-α. (B) IL-1β, (C) IL-6 and (D) IL-10 in diabetic animals. Data were expressed as Mean± SEM (n=6) and statistical analyzed using one-way ANOVA and Student-Newman-Keuls multiple comparisons test as a post hoc test. Statistical significance was considered when *P < 0.05, **P < 0.01 and ***P < 0.001. (a) D group Vs C group. 'a'D group Vs C group and 'b lutein treated groups Vs D group.

Histopathological investigation of the control group revealed normal features with no nephrotoxicity or

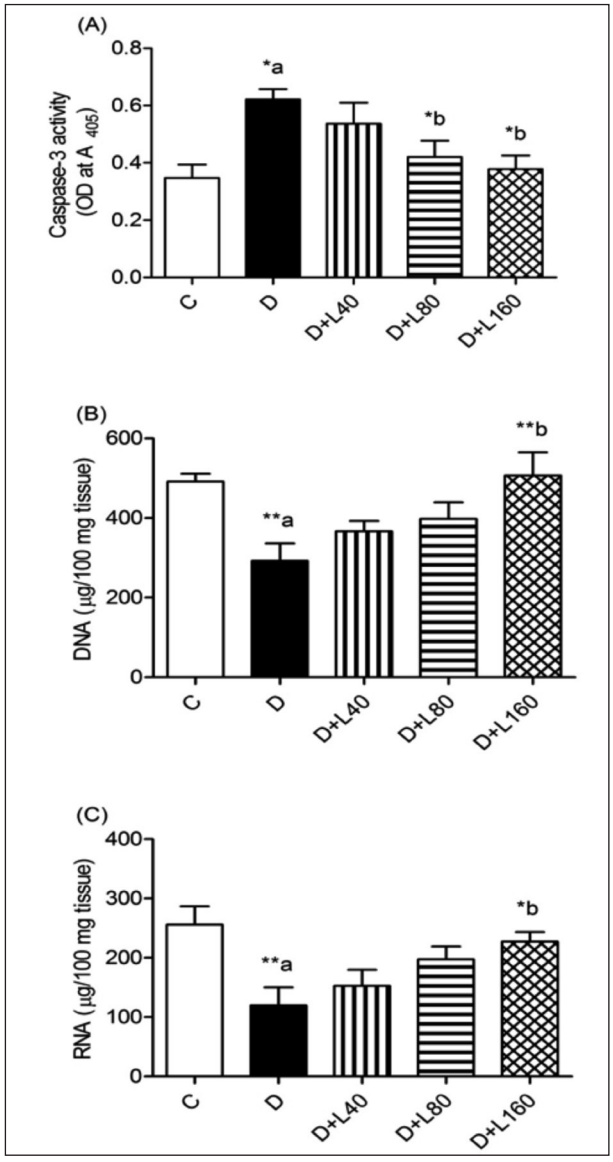

Figure 2. Effect of lutein supplementation on renal apoptosis and cytotoxicity biomarkers including (A) caspase-3, (B) DNA and (C) RNA in diabetic animals. Data were expressed as Mean ± SEM (n=6) and statistical analyzed using one-way ANOVA and Student-Newman-Keuls multiple comparisons test as a post hoc test. Statistical significance was considered when *P < 0.05 and **P < 0.01. 'a'D group Vs C group and 'b lutein treated groups Vs D group.

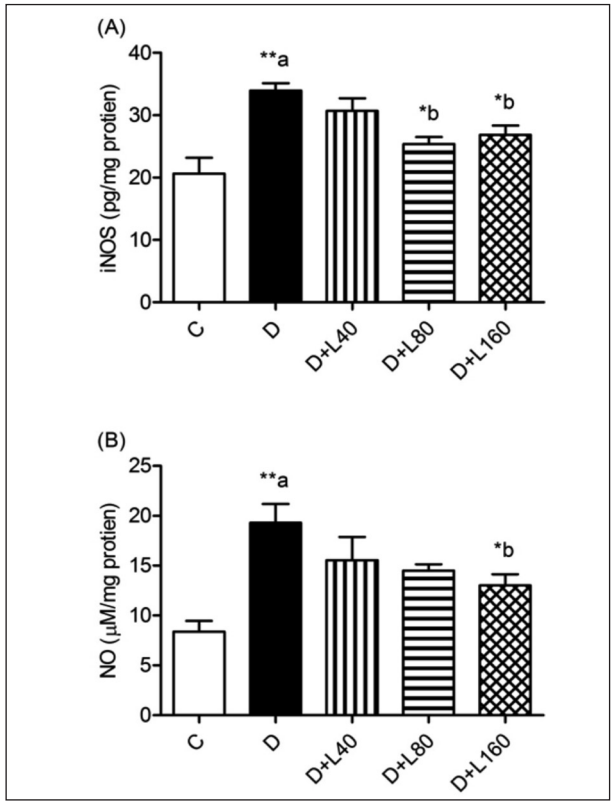

Figure 3. Effect of lutein supplementation on renal nitrosative stress biomarkers including (A) iNOS and (B) NO in diabetic animals. Data were expressed as Mean ± SEM (n=6) and statistical analyzed using one-way ANOVA and Student-Newman-Keuls multiple comparisons test as a post hoc test. Statistical significance was considered when *P < 0.05 and **P < 0.01. 'a'D group Vs C group and 'b lutein treated groups Vs D group.

glomeruli damage or interstitial nephritis (Fig. 5). In diabetic animals severe nephrotoxicity (score 8-10) with 51-75% of the damaged glomeruli and renal tubules were observed. Leucocytes were also seen within the interstitial and the tubular epithelium (Fig. 5).

The diabetic rats supplemented with lower dose of lutein showed almost similar histopathological values as observed in normal diet fed diabetic rats (score 8-10) (Fig. 5). In the lutein intermediate (80 mg/kg) dose group, 51-75% of glomeruli damage and lower renal inflammation with histopathogical diagnosis of moderate nephrotoxicity were observed (score 5-7) (figure 5). However, in higher dose of lutein group, histopathological analysis of kidney sections found mild nephrotoxicity (score 2-4) characterized by 25-50% glomeruli damage with less than 25% renal tubules were involved and mild leucocytes infiltrate (Fig. 5).

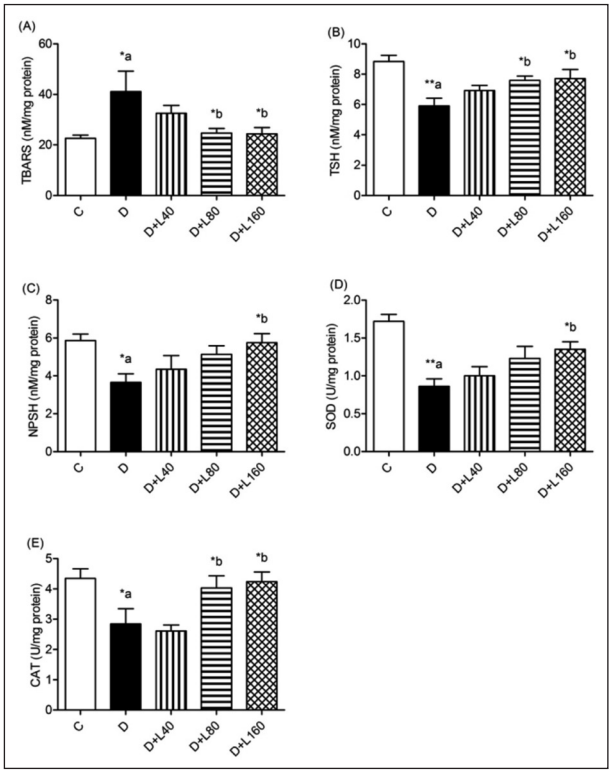

Figure 4. Effect of lutein supplementation on renal oxidative stress biomarkers including (A) TBARS, (B) TSH, (C) NPSH, (D) SOD and (E) CAT in diabetic animals. Data were expressed as Mean ± SEM (n=6) and statistical analyzed using one-way ANOVA and Student-Newman-Keuls multiple comparisons test as a post hoc test. Statistical significance was considered when *P < 0.05, **P < 0.01 and ***P<0.001. 'a'D group Vs C group and 'b lutein treated groups Vs D group.

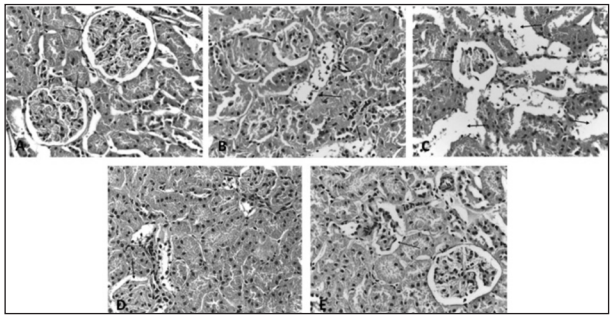

Figure 5. Effect of lutein supplementation on histopathological features of the kidneys of diabetic animals (x40). (A) Normal renal histopathological features in control animals, (B) Severe nephrotoxicity associated with glomeruli damage and inflammation in diabetic rats; (C) severe inflammatory nephrotoxicity and glomeruli and tubules injury in diabetic rats treated with low dose of lutein; (D) Moderate nephrotoxicity with partial association of glomeruli and mild inflammation in diabetic rats treated with median dose of lutein; (E) Mild nephrotoxicity without significant glomeruli damage and inflammatory infiltrate in diabetic rats treated with higher dose of lutein.

Discussion

Diabetic nephropathy is a serious complication in both types of diabetes. In our experimentally-induced diabetic animal model, mean body weights markedly decreased in diabetic rats. Diabetic associated weight loss in animals is explained by muscle wasting and loss of tissue proteins (22). Although lutein did presumably affect the metabolic status of the diabetic animals Five weeks of lutein diet supplementations to the diabetic animals could not restore the hyperglycemia associated weight retardation, which may be explained by the lack of lutein glycemic control in our study. Diabetic nephropathy also involves progressive development of hyperglycemia, hyperalbuminuria, glycosuria and proteinuria as well as a decline in glomerular renal function and renal hypertrophy (22, 23). In the current study, we noticed a marked rise in the relative kidney weights the diabetic rats compared to the controls. Increasing the kidney weights is a precise marker of renal hypertrophy and directly related to kidney inflammation (24). Lutein administration could not markedly improve the relative diabetic kidney weight increase. However, lutein supplementation was able to ameliorate the elevated levels of serum and urinary kidney function tests (urea and creatinine) indicating lessening of the diabetes-associated renal dysfunction. The nephroprotective properties of lutein were further confirmed by the histopathological investigation, where lutein supplementation, specially the higher dose, improved glomerular and tubular diabetes-induced damage and renal leucocytes inflammatory infiltrates.

Pro-inflammatory mediators including TNF-α, IL-1β and IL-6 play vital roles in the pathogenesis, development and progression of diabetic nephropathy (5). These mediators modulate the immunological and inflammatory responses during the burden of diabetic nephropathy possibly via inhibition of insulin secretion and induction of renal necrosis and apoptosis (25). TNF-α exaggerates reactive free radicals generation leading to cytotoxic effects on glomerular, epithelial and

mesangial cells. IL-1β also promotes intraglomerular hemodynamics abnormalities in mesangial cells during nephropathy (26). IL- 6 disturbs the cellular proliferation and extracellular matrix dynamics in mesangial cells and induces the thickening of glomerular basement membrane and diabetic nephropathy lesions (6). Inhibition of these inflammatory mediators markedly attenuates diabetic nephropathy progression. However, IL-10 is an anti-inflammatory cytokine that enhances the humoral immunological responses (25). Present findings indicated that diabetic nephropathy was associated with renal inflammation as the levels of TNF-α, IL-1β and IL- 6 were significantly elevated, while IL-10 was inhibited in diabetic rats' renal tissues. Dietary supplementation of lutein with different doses markedly reduced renal inflammatory proses as it decreased the levels of TNF-α, IL-1β and IL-6, while increased the levels of IL-10. Such anti-inflammatory properties of lutein were described in earlier studies (14, 27). Lutein's anti-inflammatory functions were also explained by its ability to inhibit C-reactive protein and soluble intercellular adhesion molecule (28-29) and to regulate proteasome over activity induced by oxidative stress (30). Moreover, lutein was found to suppress NF-κB activation leading to inhibition of the downstream effectors of inflammatory mediators signaling (31).

Present data revealed that, STZ injection induced significant damage in renal tissue, cellular apoptosis, cytotoxicity and formation of DNA adducts and strand breaks, was also reported in earlier studies (32-33), indicated by triggered caspase-3 activity and reduced nucleic acids (DNA and RNA) levels in kidneys of diabetic animals. Signs of cellular apoptosis, cytotoxicity were markedly attenuated by lutein diet supplementations in the diabetic animals. Such antiapoptotic and cytoprotective effects of lutein show its ability to stabilize cellular membrane peroxidation by preventing their damage and loss of important components. Earlier work of Gao *et al* also demonstrated lutein's ability to block H_2O_2-induced protein oxidation, LPO and DNA damage in lens epithelial cells (34).

DM is characterized by excessive oxidative and nitrosative stresses in different biological tissues. Alteration in the normal balance between antioxidant defense mechanisms and ROS/RNS production in favor of the latter induces oxidative/nitrosative stress leading to damage of important cellular components, which is implicated in the pathogenesis of DM. The ROS are derived from oxygen free radicals such as superoxide ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), while RNS are products of nitrogen free radicals including nitric oxide ($NO^{\bullet}$), peroxynitrite anion ($ONOO^-$) and nitrogen dioxide ($NO_2^{\bullet}$) (7). The burden of oxidative stress during DM involves a decline in the endogenous antioxidant capacity, which provokes free radicals injurious effects and LPO. Present and our earlier study demonstrated the elevated levels of LPO byproducts (expressed as TBARS) in diabetic kidneys of rodents (22). Almost all biological organs including kidneys have both non-enzymatic and enzymatic defense systems against the deleterious effects of ROS and RNS. Sulfhydryl groups including T-SH and NP-SH are important intracellular antioxidants that have a vital role in the process of free radicals removal via modulation of the cellular redox status (22). Consumption of these crucial compounds, also noticed in present study by showing one of the pathological results of hyperglycemia-induced oxidative stress and thus their levels have been considered as an indirect sign of tissue oxidative injury (35). SOD and CAT are crucial antioxidant enzymes that counterbalance the harmful devastating effects of ROS and RNS. Free radicals such as $O_2^{\bullet-}$ are reduced to H_2O_2 by the action of SOD, while CAT promotes H_2O_2 conversion to H_2O and molecular oxygen (7). Several reports, including this study, showed that intracellular hyperglycemia decreases the antioxidant enzymes activities along with LPO development, which could be attributed to glycation of these enzymes (35-36). Nitrosative stress and the synthesis of NO are reported to be increased in the kidneys of diabetic rodents, which are suggested as a vital key player in diabetes-induced nephropathy and renal hyperperfusion (37). STZ also liberates RNS along with ROS during its intracellular metabolism (22). In our study, nitrosative stress was developed in the diabetic animals kidneys as the expression of iNOS was found to be significantly higher, which lead to a greater production of NO. Lutein supplemented rats revealed a marked improvement in the diabetic induced inhibition of renal TSH and NPSH levels and SOD and CAT activities along with ameliorated LPO. Moreover, lutein supplemen-

tation attenuated nitrosative stress representing free radical scavenging activity and renal protective ability. Lutein's renal protective effects against oxidative injury are in accordance with earlier studies, which proposed lutein's beneficial effects following intake due to its antioxidative potentials (14, 27). The antioxidant property and most of the biologic functions of lutein are attributed to its chemical structure, which contains two hydroxyl groups, one on each side of the molecule (38-39) making it a strong quencher of ROS and RNS and explains its ability to scavenge the generated free radicals (15, 27). Using the same experimental model of diabetes, Sasaki and his co-workers proved that the antioxidant properties of lutein via prevention of oxidative stress and ROS generation improved the visual impairment in the diabetic retina. (40) Recently, Sindhu and Kuttan reported that lutein treatment to cisplatin challenged mice inhibits malondialdehyde levels and enhances the enzymatic activities of SOD and CAT in renal tissue (41). In another recent study, Liu *et al* demonstrated that lutein has a significant protective effect against ischemia-reperfusion injury by enhancing antioxidant defense mechanisms (42).

Conclusion

The present study suggests the therapeutic value of lutein to reverse diabetic renal injury and inflammation via its anti-apoptotic and free radical scavenging actions. However, further studies are needed to elucidate the clinical significance of lutein supplementation and its related nephro-protective actions. This study also emphasizes the importance of basic experimental research using animal models to understand different actions and mechanisms occurring during diabetic nephropathy.

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