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Ameliorative effect of lycopene alone and in combination with co-enzyme Q10 in streptozotocin-induced diabetic nephropathy in experimental rats

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ABSTRACT

Diabetic nephropathy (DN) has become an utmost reason for long-standing renal dysfunction and end-stage renal disease globally. Oxidative stress induced by persistent hyperglycemia is considered a fundamental element in the evolution of DN. The goal of this research was to discover the outcome of appendages of natural antioxidants such as lycopene and co-enzyme Q10 (CoQ10) in DN rats and to observe the preventive effects in DN. A diabetes model was developed in a Wistar strain of male rats (200–250 g) by subcutaneous injection of streptozotocin (55 mg/kg). Development of nephropathy was assessed by renal function tests including blood glucose, creatinine, albumin, total protein, total bilirubin, uric acid, total cholesterol, triglycerides, and CRP level. Oxidative stress markers such as LPO and GSH content and activity of membrane-bound Na^+/K^+ ATPases were measured in kidney homogenate. Renal damage was assessed by performing a histopathological analysis. DN rats showed a significant elevation in creatinine, albumin, total protein, total bilirubin, uric acid, total cholesterol, triglycerides, CRP, and LPO levels whereas a significant reduction in creatinine clearance and GSH level. Treatment with antioxidants such as lycopene (5 mg/kg/p.o.) and CoQ10 (10 mg/kg/p.o.) along with their combination for 4 weeks notably altered the level of renal function biomarkers and oxidative stress markers. These antioxidants and their combination also protected the kidney from abnormal morphological changes. The present findings suggest that the combined administration of lycopene and CoQ10, which are antioxidants, exhibits synergistic effects in mitigating renal injury by reducing hyperglycemia, oxidative stress markers, and histopathological alterations.

1. Introduction

Diabetes mellitus (DM) patients have poor glycemic control and experience some microvascular consequences involving diabetic nephropathy (DN) (Balakumar et al., 2009; Marcovecchio et al., 2011). DN is specified by anatomical and physiological abnormalities in the kidney (Forbes et al., 2008).

The hallmarks of DN comprise buildup of extracellular matrix, stiffness of the basal membrane, scarring of the glomerulus, podocyte mislaid, enlargement of mesangial cells, and tubular abnormalities (Arora & Singh, 2013; Horie et al., 1997; Ritz, 2013). These modifications lead to changes in urinary albumin excretion, glomerular filtration rate (GFR), microalbuminuria, uremia, creatinine clearance rate, etc. Cumulatively all the above alterations in pathogenesis lead to End-Stage Renal Disease (ESRD) (Ritz et al., 2011; Schena & Gesualdo, 2005; Sudamrao Garud & Anant Kulkarni, 2014).

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Hyperglycemia, hyperlipidemia, and proteinuria are the key players that cause renal injury in DN. Hyperglycemia triggers many renal cells for the stimulation of humoral mediators and cytokines. These substances lead to many changes in the kidney such as increased ECM deposition, buildup of advanced glycation end products (AGEs), and alteration in permeability of the basal membrane which promotes glomerular fibrosis and aggravates DN (Schena & Gesualdo, 2005).

Oxidative stress (OS) markedly takes part in the genesis of DN and hyperglycemia-produced oxidative stress is strongly indicated in various studies (Ighodaro, 2018; Kashihara et al., 2010). Elevated OS in a hyperglycemic state causes activation of various pathways such as P38-MAPK, AKT, and Rheb pathway which promote fibrosis and inflammation of tissue in the kidney that is attributable to DN (Bhattacharjee et al., 2016; Fakhruddin et al., 2017; Platé et al., 2020).

Reports suggested that anti-oxidative therapy has shown promising results in reducing and delaying the signs and symptoms associated with animal models of streptozotocin-induced DN in rats (Akbar et al., 2011; Mahmoodnia et al., 2017; Rojas-Rivera et al., 2012; Tavafi, 2013).

Lycopene is a bioactive carotenoid, generally found in tomatoes. It is a powerful oxygen quencher among other carotenoids and could effectively lower the formation of reactive oxygen species (ROS) (Shi et al., 2004; Zhu et al., 2020). It reduces the likelihood of developing many chronic diseases including cardiovascular disorders (Sayahi & Shirali, 2017), diabetes (Zhu et al., 2020), anti-inflammatory (Puah et al., 2021), kidney diseases (Zhu et al., 2011) and asthma (Leh & Lee, 2022).

Another important antioxidant is Coenzyme Q10 (CoQ10), an internally produced hydrophobic substance, commonly found in mitochondria and essential in ATP production during oxidative phosphorylation (Hodgson et al., 2002; Mahajan et al., 2020). It inhibits the peroxidation of protein and lipid along with free radical scavenging action and thus, guards tissue from oxidative injury (Rosenfeldt et al., 2007).

Several studies have revealed that the combination of antioxidants shows synergistic effects in the conditions such as myocardial infarction (Upaganlawar & Gandhi, 2010), inflammation (Fuller et al., 2006), hepatorenal toxicity (Elsayed et al., 2021), neuropathy (Esu et al., 2022), migraine (Parohan et al., 2021), prostate cancer (Gunasekera et al., 2007), atherosclerosis (Li et al., 2019), and autoimmune disease (Vetvicka & Vetvickova, 2018). Considering the available literature, no study to date investigated the concomitant results of lycopene and CoQ10 in DN. The current work was outlined to assess the effectiveness of lycopene and CoQ10 combination in diabetic nephropathy in laboratory rats.

2. Materials and methods

2.1. Drugs and chemicals

Lycopene and CoQ10 were obtained from Universal Industries, Nashik, and Zydus Cadila, India respectively. Streptozotocin (STZ) was purchased from Sigma Aldrich (USA). All chemical and necessary diagnostic kits were of standard class.

2.2. Experimental animals

Healthy male rats of the Wistar strain were split into five groups each consisting of six rats. The rats were obtained from Wockhardt Ltd. Aurangabad, India. They were housed as per CPCSEA guidelines. The study methods were permitted by the IAEC of the organization, (SSDJ/IAEC/2021-22/03).

2.3. Induction of DN using STZ

DN was developed by a single dose of STZ (55 mg/kg) prepared in 0.2 ml of citrate buffer (0.1 M, pH 4.5). Diabetes was confirmed 72 h after the STZ dosing and the blood glucose level (BGL) was measured by digital Glucometer (ACCU-CHEK, Roche Diabetes Care, Germany). Then rats were tested for four weeks for the progression of nephropathy.

2.4. Experimental design

Five groups of rats were formed, with each group comprising six rats. The descriptions of the groups are as follows:

Group I: Control rat treated with vehicle alone (0.2 ml/kg/s.c.)
Group II: Rats administered with STZ (55 mg/kg/s.c.) (Mahajan et al., 2020)
Group III: Rats treated with lycopene (5 mg/kg/p.o./day) dissolved in distilled water (Pansare et al., 2021)
Group IV: Rats treated with CoQ10 (10 mg/kg/p.o./day) (Mahajan et al., 2020)
Group V: Rats treated with lycopene (5 mg/kg/p.o./day) plus CoQ10 (10 mg/kg/p.o./day) simultaneously in combination

All drug treatment was given for 4 weeks.

2.5. Estimation of body weight, kidney weight, and kidney hypertrophy index

In the 4th week, rats were sacrificed and kidneys were removed. The body and kidney weight of the rats were measured by electronic weighing balance and the kidney index was evaluated as per the given formula (Liu et al., 2010):

$$Kidney\ hypertrophy\ index\ (\%) = \frac{Kidney\ weight}{Body\ weight} x\ 100$$

2.6. Estimation of biochemical parameters in serum and urine

In the 4th week of the study, rats were individually kept in metabolic cages for one day, and urine sample collection was done. Blood was obtained from the retro-orbital nerve by employing ether anesthesia using micro-capillary and the serum was separated using high-speed centrifugation. Urine and serum samples were kept at –20 °C and used for various biochemical estimations. Blood glucose was measured using a glucometer. Total protein, total bilirubin, uric acid, total cholesterol, triglycerides, and C-reactive protein (CRP) were analyzed in serum and the level of albumin and creatinine were determined in urine samples using commercial diagnostic kits. Creatinine clearance was calculated by the given formula (Lavender et al., 1969):

$$Creatinine\ clearance\ \left(\frac{ml}{min}\right) = Urinary\ creatinine\ x\ \frac{Urine\ volume}{Serum\ creatinine}$$

2.7. Tissue homogenization

At the end of treatment, the rats were sacrificed via the decapitation method and the kidney was excised, and washed in cold physiological saline. One kidney was stored for histopathological examination in 10% formalin solution while the other kidney was used for the estimation of antioxidant enzyme. The kidney was finely chopped into small pieces in a chilled sucrose solution (0.25 M). The renal tissues were homogenized in 10% w/v *tris*-hydrochloride buffer (10 mM, pH 7.4). The homogenate was centrifuged at 10000 rpm for 15 minutes at 0 °C using a cooling centrifuge (Jain, 2015). The supernatant was utilized to determine the concentration of lipid peroxidation (LPO) and reduced glutathione (GSH), while the assessment of Na^+/K^+ ATPases was conducted using a sediment-based method.

2.8. Evaluation of OS markers

2.8.1. Determination of lipid peroxidation (LPO)

2.0 ml homogenate was mixed with 2.0 ml of 10% (w/v) TCA and cooled for 15 minutes. The precipitate was separated by centrifugation. 2.0 ml of thiobarbituric acid (TBA) was added to a clear supernatant and the solution was heated. The pink color formed by the reaction of TBA with MDA was read at 532 nm against the blank. Standard malondialdehyde in a different concentration (0-23 nM) was used. The values were expressed as nM of MDA/mg protein (Slater & Sawyer, 1971).

2.8.2. Determination of reduced glutathione (GSH)

The deproteinization of kidney homogenate (supernatant) was made by using 20% TCA and then centrifugation was performed. 0.25 ml of supernatant, 2 ml of DTNB reagent, and phosphate buffer were added. The yellow color produced was measured at 412 nm. Different concentrations (10-50 µg) of standard glutathione were prepared. The concentration of GSH was mentioned as GSH mg/g protein (Moron et al., 1979).

2.8.3. Determination of Na^+/K^+ ATPase

In 0.2 ml homogenate, 1 ml of *tris*-hydrochloride buffer, and 0.2 ml each of sodium chloride, magnesium sulfate, EDTA, potassium chloride, and ATP were added. The mixture was incubated for 15 minutes at 36 °C. The reaction was arrested by 1.0 ml of TCA (10%), and centrifuged. The phosphorus content of the solution was measured and the enzymatic activity was expressed as nM of IP liberated/gm protein/min (Lowry et al., 1951).

2.9. Histopathological examination

A 5 µm portion was prepared, after which the sections were stained with hematoxylin and eosin (H&E), and assessed for general morphological alteration under the light microscope (400× magnification) and the photographs were taken.

2.10. Statistical analysis

All variables were analyzed by Graph Pad Prism (version 5.01), after implementing ANOVA with Dunnett's post-hoc test. Data was given in terms of mean ± standard error of mean (SEM) (n = 6). Among all tested groups, a statistical difference at the level of $p < 0.05$ was considered statistically significant.

3. Results and discussion

Antioxidants have been investigated for their potential therapeutic effects in diabetes and DN. Antioxidants are substances that reduce oxidative stress and thereby free radical production. Hyperglycemia leads to renal tissue damage by modifications of many hemodynamic factors (Ashrafi et al., 2017). Oxidative stress generated during DN is responsible for the apoptosis of renal tissue (Kukner et al., 2009).

The injection of STZ into the experimental rats in this study resulted in permanent diabetes due to the toxic effects of STZ on the pancreatic beta cells, leading to their dysfunction and subsequent disruption of insulin production, and, in turn, contributed to the development of hyperglycemia and OS in the renal tissue (Daniel et al., 2015; Wang et al., 2016).

Hyperglycemia is a vital tool for monitoring DN development (Wang et al., 2016). As indicated in Figure 1, at the end of the 4th week, fasting BGL was estimated. DN rats demonstrated markedly elevated levels of BGL ($p < 0.001$) as compared to control rats indicating severe destruction of beta cells. Administration of lycopene and CoQ10 alone as well as in combination markedly reduced the BGL ($p < 0.001$) compared to DN rats by inhibiting activation of hemodynamic pathways. The result was matched with the formerly reported research work (Pansare et al., 2021).

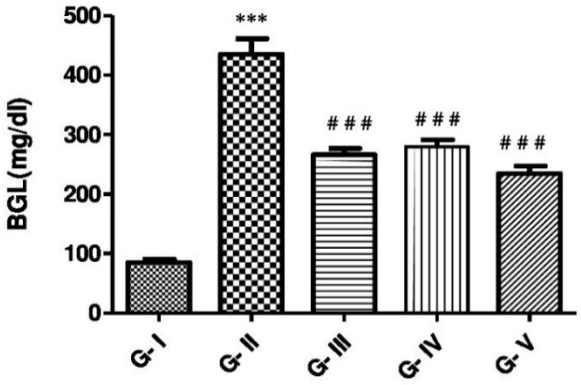

Figure 1. Lycopene, CoQ10, and its combined effects on BGL
Data was mentioned in terms of mean ± SEM (n=6).
ANOVA was applied with the Dunnett t-test for analysis.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to normal and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to DN.

STZ-induced diabetic rats showed a remarkable loss in body weight. Depletion in body weight after STZ injection might be due to the oxidation of tissue proteins, disruption of muscle tissue, and gluconeogenesis (Daniel et al., 2015). It is considered that renal hypertrophy is the main pathological indicator of diabetes-induced renal dysfunction (Ghule et al., 2012). In the present research, the kidney hypertrophy index of STZ-treated rats was notably raised due to alteration in kidney and body weight as compared with normal rats ($p < 0.01$). This could be due to structural damage to renal tissue. It is previously reported that kidney hypertrophy has been linked to increased kidney weight in DM because of excessive usage of glucose, and an elevated synthesis of protein and phospholipids in the renal tissue (Mogensen, 2004; Teoh et al., 2010). In DN rats treated with lycopene, the kidney hypertrophy index was markedly decreased as expected ($p < 0.05$). When compared to the STZ rats, rats treated with the combination of CoQ10 and lycopene showed

markedly decreased levels of kidney hypertrophy index ($p < 0.01$) than rats treated with only lycopene (Figure 2).

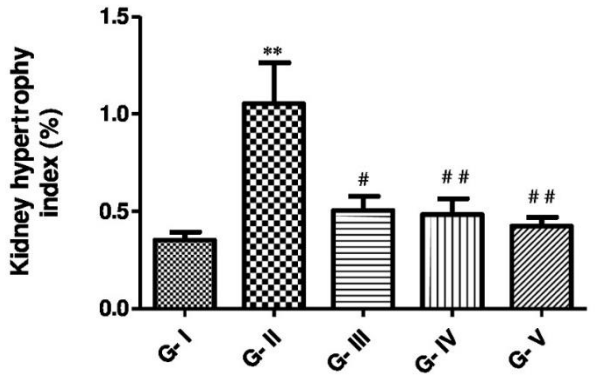

Figure 2. Lycopene, CoQ10, and its combined effects on kidney hypertrophy index

Data was mentioned in terms of mean ± SEM (n=6).
ANOVA was applied with the Dunnett t-test for analysis.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to normal and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to DN.

Long-term hyperglycemia leads to polyuria in DM. In the current research, it was noticed that DN rats displayed a remarkable increase in urine volume (24 h) ($p < 0.001$). It was demonstrated that the level of renal function biomarkers i.e. creatinine, albumin, total protein, total bilirubin, and uric acid were notably increased in DN rats ($p < 0.001$) indicating abnormalities in the excretory and regulatory function of the kidney leading to interstitial atrophy, necrosis of epithelial cells due to oxidative damage (Akinnuga et al., 2019; Lee & Ku, 2008; Nakagawa et al., 2005; Yokozawa et al., 2005). These alterations in biomarkers level were due to the accumulation of glucose in blood and urine (Sangar & Singh, 2016). Microalbuminuria and creatinine are the key indicators of renal failure. DN rats showed remarkable reduction in creatinine clearance due to damage to the tubule cells ($p < 0.001$). The present reports indicated that DN rats treated with antioxidants lycopene and CoQ10 showed markedly decreased levels of urine output ($p < 0.01$), renal biomarkers such as creatinine, albumin, total protein, total bilirubin, and uric acid whereas rats administered with their combination showed a marked reduction in these levels ($p < 0.001$) compared with single treatments with these agents. The creatinine clearance also increased significantly after treatment with both antioxidants ($p < 0.001$) (Table 1).

Table 1. Lycopene, CoQ10, and its combined effects on biochemical parameters

Parameters	Group I	Group II	Group III	Group IV	Group V
Urine volume (ml/day)	24.4 ± 1.04	90.0 ± 1.45***	59.0 ± 2.39###	61.6 ± 1.43###	45.5 ± 1.93###
Urine creatinine (mg/dl)	1.09 ± 0.15	3.31 ± 0.40***	1.85 ± 0.33#	1.76 ± 0.27#	1.39 ± 0.31##
Creatinine clearance (ml/min)	1.49 ± 0.14	0.30 ± 0.03***	0.66 ± 0.04###	0.79 ± 0.03###	1.10 ± 0.06###
Urine albumin (g/dl)	1.66 ± 0.115	4.68 ± 0.210***	3.51 ± 0.185##	2.93 ± 0.265###	2.43 ± 0.170###
Total protein (g/dl)	6.79 ± 0.651	13.81 ± 1.073***	9.69 ± 0.998##	10.08 ± 0.663#	8.35 ± 0.642###
Total bilirubin (mg/dl)	0.81 ± 0.20	2.31 ± 0.37**	1.08 ± 0.26##	1.31 ± 0.16#	1.07 ± 0.15##
Uric acid (mg/dl)	2.21 ± 0.244	7.77 ± 0.296***	5.53 ± 0.415##	5.33 ± 0.445##	4.36 ± 0.479###
Total cholesterol (mg/dl)	75.44 ± 4.87	144.3 ± 4.59***	112.5 ± 6.64##	106.4 ± 6.67###	83.07 ± 2.87###
Triglyceride (mg/dl)	71.33 ± 7.97	165.3 ± 4.61***	107.3 ± 2.95###	110.1 ± 4.01###	92.63 ± 2.38###

Data was mentioned in terms of mean ± SEM (n=6).
ANOVA was applied with the Dunnett t-test for analysis.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to normal and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to DN.

It was found that chronic hyperglycemia causes significant dyslipidemia in DN rats due to abnormal insulin regulation ($p < 0.001$). Insulin deficiency causes high serum lipid levels by the mobilization of free fatty acids. It also hydrolyzes triglycerides by stimulating lipoprotein lipase (Raghunathan et al., 2014; Wang et al., 2011). Research has proclaimed the relationship between aberrant lipid levels and the risk of the succession of DN (Marcovecchio et al., 2011). In this investigation, increased levels of total cholesterol and triglycerides in diabetic rats were remarkably lowered with the 4-week therapy of lycopene, CoQ10 alone, and a combination of both, which may be added to its renoprotective effects in DN ($p < 0.001$) (Table 1).

CRP appears in blood during an inflammatory process, which increases significantly in diabetes conditions ($p < 0.001$) due to tissue injuries. It was found that after the treatment, lycopene significantly declined the CRP level ($p < 0.01$). STZ-exposed rats treated by CoQ10 alone remarkably showed reduced levels of CRP ($p < 0.05$). Whereas simultaneous treatment with lycopene and CoQ10 of DN rats more remarkably declined the CRP level than single treatments ($p < 0.001$) (Figure 3).

STZ-treated animals show declined defense mechanisms of antioxidants in OS (Orhan et al., 2006) (Figure 4). In this investigation, excessive ROS generated in DN rats led to the oxidation of biomolecules, particularly lipid peroxidation and significantly increased its level ($p < 0.001$). LPO is considered a pivotal indicator of OS. Excessive LPO is responsible for high glucose levels by damaging the beta cells (Patel et al., 2014). Antioxidants prevent the interaction between free radicals and biological substances (Arora et al., 2002). It was found that after a 4-week treatment of lycopene, CoQ10, and their simultaneous dose to the STZ rats, they demonstrated a notable reduction in LPO level as correlated with DN groups ($p < 0.001$) (Figure 4a).

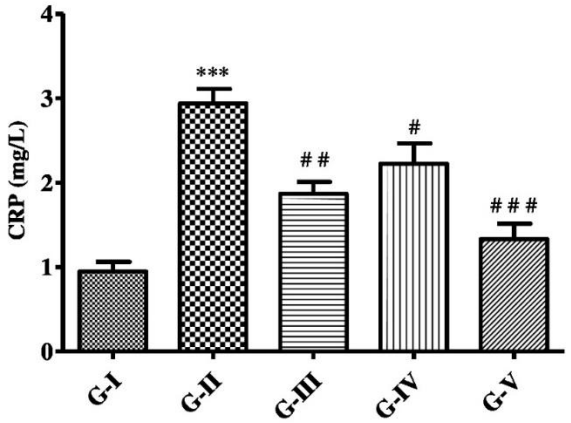

Figure 3. Lycopene, CoQ10, and its combined effects on serum CRP level

Data was mentioned in terms of mean ± SEM (n=6).
ANOVA was applied with the Dunnett t-test for analysis.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to normal and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to DN.

Reduced glutathione (GSH) is an endogenous antioxidant having a major function in numerous biological activities such as cellular growth, protein synthesis, and metal chelation (Bartoli et al., 2017) and maintains redox balance in the cells. GSH has the property to redevelop another hydrophobic antioxidant (Sharma et al., 2012). The significant decline of GSH levels in the kidney tissue of DN rats hastens OS ($p < 0.001$) (Patel et al., 2014) (Figure 4b). This result was in line with earlier recorded studies (Jain et al., 2020; Suvarchala Reddy et al., 2019). Rats that received lycopene showed a remarkable raise in GSH levels than CoQ10 ($p < 0.001$) but a combination of both showed a more beneficial elevation in GSH levels compared with the DN rats ($p < 0.001$).

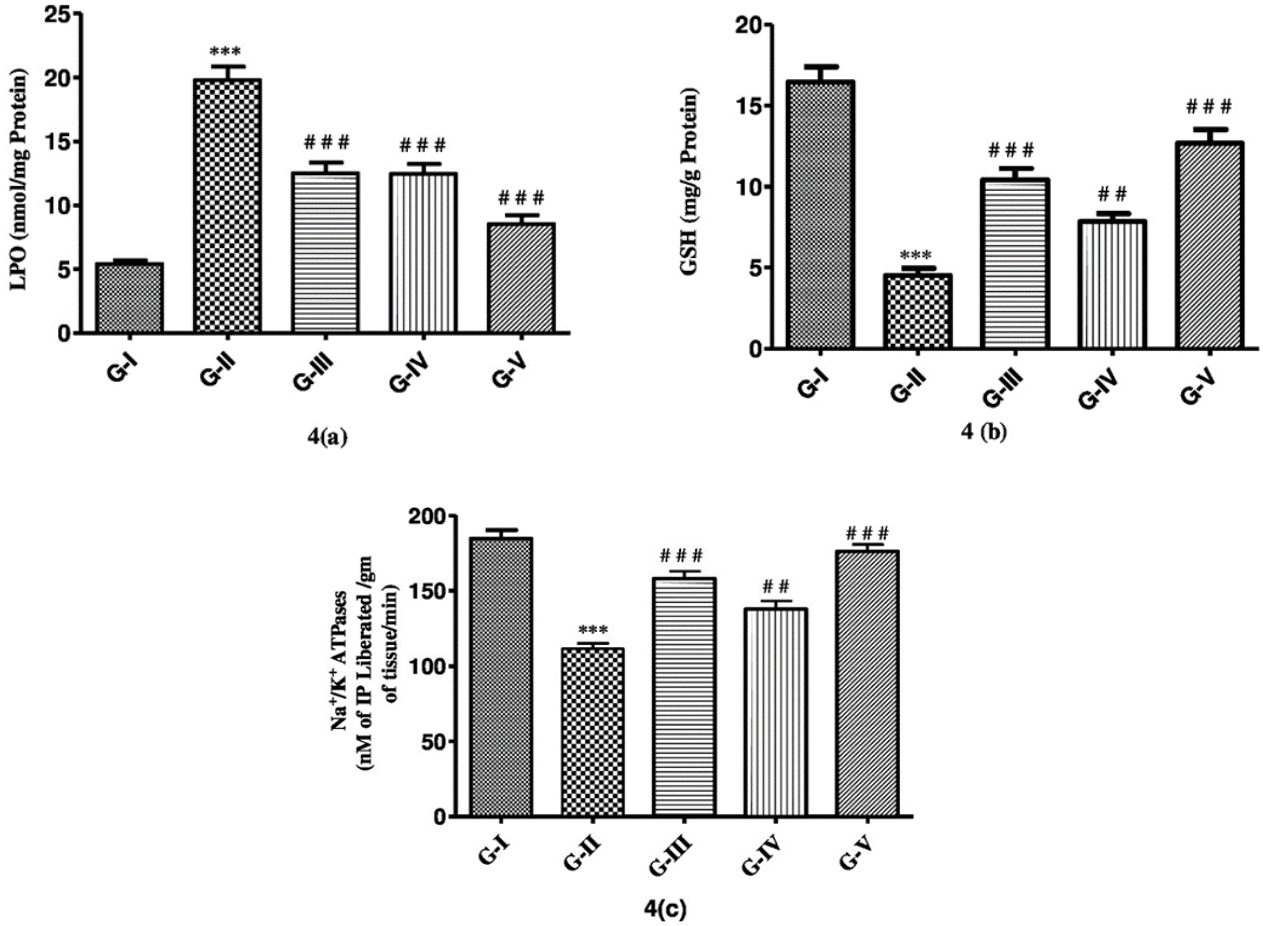

Figure 4. Lycopene, CoQ10, and its combined effects on markers of oxidative stress in the renal tissue

4(a) LPO, 4(b) GSH, 4(c) Na^+/K^+ ATPases

Data was mentioned in terms of mean ± SEM (n=6).

ANOVA was applied with the Dunnett t-test for analysis.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to normal and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to DN.

The activity of the membrane-bound ATPase i.e. Na^+/K^+ ATPase was notably declined in DN rats ($p < 0.001$) when compared with control rats. This might be due to changes in the ionic homeostasis of membrane-bound enzymes (Senthil et al., 2007). In this study, it was noticed that treatment with lycopene alone more effectively improved membrane-bound enzyme activity ($p < 0.001$) than CoQ10 but the combination exerted a more potent increase in levels of membrane-bounded enzymes ($p < 0.001$). This finding provides elucidation regarding the concurrent administration of antioxidants, highlighting their synergistic functionality when used in combination (Figure 4c).

A histopathological study of the kidney was performed to figure out whether these biochemical changes in renal function biomarkers cause structural alterations at the microscopic level. This analysis conceded that long-term diabetes was responsible for pronounced morphological alteration in the anatomy of the glomerulus and tubular cells of the kidney. It was noticed that DN groups showed structural modification such as the rise in the stiffness of the glomerular basement membrane, and degeneration of tubular cells that hastened vacuole formation in the tubule (Figure 5b). Rats administered with antioxidants such as lycopene and CoQ10 alone showed lesser glomeruli impairment with minimal tubular cell degeneration (Figure 5c and 5d). Whereas, treatment with the combination of both antioxidants more effectively decreased abnormal morphological changes in glomeruli and tubular cells of DN rats, and protected renal tissue from diabetes-associated injury (Figure 5e). The histological findings cleared that these structural changes were due to the abnormal excretory and regulatory function of the kidney and this alterations were markedly reversed by antioxidant therapy.

4. Conclusions

The present investigation suggested that natural antioxidants such as lycopene and CoQ10 can modulate hyperglycemia and oxidative stress generated due to DM. The simultaneous treatment of both lycopene and CoQ10 displayed a remarkable renoprotective effect

by improving the function of the renal antioxidant system and altering morphological changes. The findings of this investigation demonstrated that the combined effect of lycopene and CoQ10 were due to their antioxidant property which attenuate the development of DN.

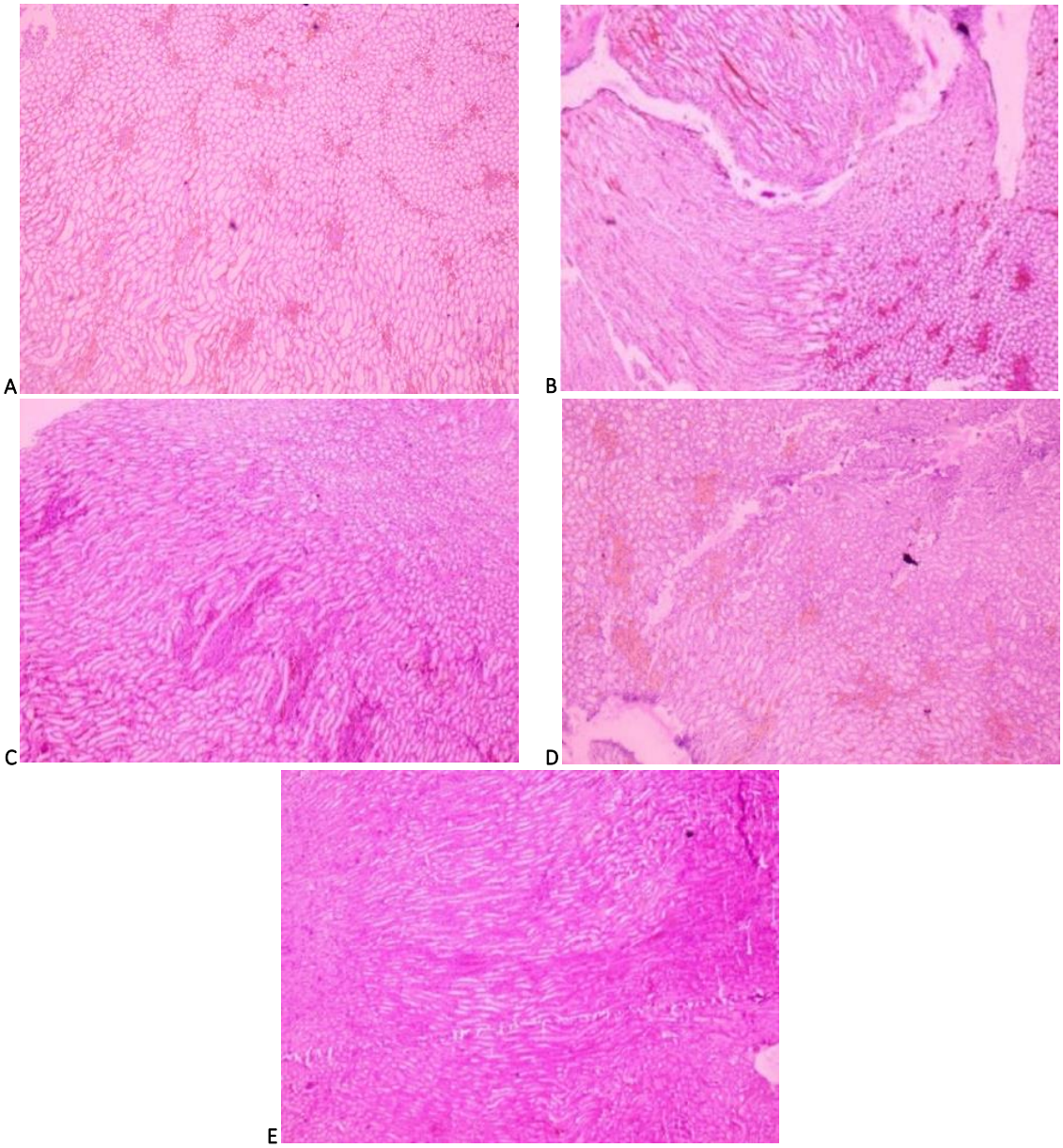

Figure 5. Photomicrographs of histopathological analysis of renal tissue of rats by H & E staining

A) Group- I, B) Group- II, C) Group- III, D) Group- IV, E) Group- V

A) Group-I: The renal tissue section of control rats showed a normal structure of the glomerulus and renal tubules.

B) Group-II: Diabetic rats showed a raised thickness of the basement membrane of glomeruli and degeneration of some tubular epithelial cells, which are prone to glomerulosclerosis and tubular atrophy.

C) Group-III: Rats treated with lycopene show lesser glomeruli impairment and tubular cell degeneration.

D) Group-IV: Tissue section showing fewer tubular vacuole formation.

E) Group V: The renal section treated with the simultaneous dose of lycopene and CoQ10 shows a normal appearance of glomeruli with improvement in tubular degeneration.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

The methods in this study was permitted by IAEC of the organization, (SSDJ/IAEC/2021-22/03) and carried according to the CPCSEA guidelines.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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CRediT authorship contribution statement

Pooja B. Rasal: Conceptualization, Investigation, Writing original draft
Gaurav N. Kasar: Conceptualization, Investigation, Writing original draft
Manoj Mahajan: Investigation, Supervision, Review and editing, Software
Aman Upaganlawar: Investigation, Supervision, Review and editing, Software
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Supplementary File

None.

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