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Original Research Article

The effect of co-enzyme Q10 and N-acetylcysteine on biochemical parameters and oxidative stress in rats subjected to alloxan induced diabetic nephropathy

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ABSTRACT

Introduction: Diabetic nephropathy (DN) has been recognized as the one of the microvascular chronic complications resulting from diabetes. Though, efforts have taken to develop effective therapies for attenuation of DN, involvement of the multiple pathogenetic mechanisms and underlying oxidative stress (OS) makes it difficult to choose optimum therapeutic agent.
Objective: The study is aimed at assessing the possible therapeutic effect of coenzyme Q10 (CoQ10) and N-acetylcysteine (NAC) individually or in combination on experimental DN in rats.
Materials and Methods: The study was performed on adult male Sprague Dawley rats (n = 50; 200–250 g). Rats were made diabetic by alloxan (130 mg/kg; i.p.). The non-diabetic rats were randomly assigned to control, CoQ10 (10 mg/kg, p.o.), NAC (300 mg/kg, p.o.), and CoQ10+ NAC group. Whereas diabetic rats were grouped as DN control, DN+ CoQ10, DN+ NAC and DN+ CoQ10 +NAC groups. CoQ10 and/or NAC were administered to the diabetic rats for 8 weeks after confirmation of the DN. Renal functions and tissue antioxidant parameters were estimated.
Results: Renal function tests and urinary proteins were significantly ($p<0.05$) higher within DN control group as compared with the diabetic groups ($p<0.05$) receiving treatments for 8 weeks. The study showed significant effect of combined treatment in improving renal function and antioxidant status in DN rats.
Conclusion: The study provides valuable information supporting that treatment with combination of CoQ10 and NAC might prevent or delay the renal damage associated with DN and improves the antioxidant.

1. Introduction

Renal function plays major role in the homeostatic maintenance body functions which is challenged during Diabetes mellitus (DM), the commonest of the systemic diseases involving the kidney. DN is one of the microvascular chronic complications resulting from DM due to chronic hyperglycemia. It is considered as the leading causes of chronic renal failure (CRF) and end stage renal disease (ESRD) and increased morbidity and mortality in the diabetics globally.[1]

Almost all diabetic complications including DN is linked to the persistent hyperglycemia. It is the major factor in diabetic microvascular complications, particularly in the kidney. Also, this chronically elevated blood glucose level is the principal player in the generation of early and sustained OS during DM. OS along with chronic hyperglycemia is involved in the pathogenesis of glomerular and tubular functional and structural abnormalities, even before the onset of microalbuminuria.[2]

The healthy kidney due to its high metabolic activity is capable of generating a considerable OS counterbalanced by an antioxidant defence. Studies showed that the significant contributor to the diabetic complications is persistent hyperglycemia that shifts this balance to a pro-oxidant state leading to tissue damage and vascular injury. It is shown that almost all pathways contributing to the DN induce oxidative stress by one or other mechanism.[3]

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Presently, the therapies available for the treatment of DN have not been fully efficacious due the involvement of numerous mechanisms contributing to the DN. It is therefore difficult to choose best therapeutic agent from the available options for attenuation DN. There are limited and only partially effective agents, thus interest in the development of new strategies is high. Therefore, effective and new therapeutic approaches than available are needed in the treatment of DN.[4]

Several studies showed that antioxidant have a beneficial effect on DN with betterment in the renal physiology by acting directly against oxidative tissue damage. Moreover, antioxidants administered exogenously protect against progression of DN.[4,5] Antioxidant do this by blocking the formation of excessive ROS effectively and scavenging the preformed intracellular ROS. Among these antioxidants, most of the exogenous antioxidants have shown advantageous effects in amelioration of kidney dysfunctions in DN.[6] Therefore, the use of antioxidants as a complementary therapy is useful in OS-related diseases including DN.[7]

None of the studies till date have shown the protective effect of the CoQ10 and NAC or mainly their combination in experimentally induced DN. Therefore, in the present study was aimed at assessing the possible therapeutic effect of CoQ10 and NAC individually or in combination on DN in alloxan (ALX) induced DM.

2. Materials and Methods

2.1. Drugs and chemicals

Coenzyme Q10 was obtained as a gift sample from Zydus Cadila, Ahmedabad, India. N-acetylcysteine was procured from Loba Chemie (Mumbai, India). ALX was purchased from Sigma-Aldrich (USA). Spectrophotometric kits for assessment of malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) were purchased from Elabscience Biotechnology Inc. (Houston, USA). Biochemical kits for estimation of total protein, and albumin were purchased from Arkray Healthcare Pvt. Ltd. (Mumbai, India). Kits for estimation of creatinine and BUN, used in the study were purchased from Tulip Diagnostics Pvt. Ltd. (Mumbai, India). All other chemicals and reagents used in the study were of analytical grade.

2.2. Experimental animals

The study was conducted using adult male Sprague-Dawley rats (8 weeks, 220-250 g). Animals were procured from the National Institute of Bioscience, Pune and housed under standard conditions in polypropylene cages. Rats were housed in a controlled environment of temperature (18-22ºC) and light (12-hr light/dark cycle, lights on 07:00-19:00). All animals were given ad libitum access to standard food and water and were acclimated for 1 week prior to the beginning of the study. All the procedures which applied to rats in this work were performed according to the ethical guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, on the care and use of animals. The protocol of the study was approved by Institutional Animal Ethics Committee (IAEC) bearing the reference number SSDJ/IAEC/2016/02-02.

Type I DM was induced in overnight fasted rats by single intraperitoneal injection of ALX monohydrate (130 mg/kg, i.p.). ALX was administered immediately after dissolving it in freshly prepared cold citrate buffer, (pH 4.5). Hyperglycemia was assessed at 72 h and then on day 7 post-ALX injection by determination of blood glucose level using glucometer (AlereG1, Korea) in peripheral samples gathered from the tail vein. Animals with blood glucose level greater than 250 mg/dL were considered as diabetic and included in the DN studies.

2.3. Experimental design

Non-diabetic and ALX diabetic rats (animals with blood glucose level greater than 250 mg/dL were considered as diabetic and included in the DN studies) were randomly allocated into 8 groups with 6 animals each:

Group I: Normal control rats.

Group II: CoQ10: Non-diabetic rats treated with10 mg/kg CoQ10 suspended in 1% aqueous solution of tween 80, p.o.[8,9]

Group III: NAC: Non-diabetic rats treated with NAC, 300 mg/kg, p.o dissolved in distilled water.[10,11]

Group IV: CoQ10+NAC: Non-diabetic rats treated with combination of CoQ10 and NAC Group V: ALX-DN: Diabetic control rats

Group VI: DN+CoQ10: Diabetic rats treated with CoQ10

Group VII: DN+NAC: Diabetic rats treated with NAC

Group VIII: DN+CoQ10+NAC: Diabetic rats treated with a combination of CoQ10 and NAC.

Treatments of Diabetic animals were initiated at the beginning of 5^{th} week and continued till the end of 12^{th} week of the study duration.

At the end of the protocol, and twenty-four hours after the last antioxidant dose, urine samples were collected by placing the animals individually in metabolic cages for 24-hours. The blood samples were collected from retro-orbital plexus under anesthesia.

HbA1C was estimated using whole blood. Total protein, albumin and creatinine were estimated in serum and urine samples. BUN was estimated from serum using standard diagnostic kit with the help of Prietest Touch Biochemistry Analyzer, Robonik India Pvt Ltd. (Mumbai, India).

Creatinine clearance (Ccr, mL/min) was determined from the following formula: (urine creatinine [mg/dL] ×24h urine volume [mL])/ (serum creatinine [mg/dL]×

1440 [min]). UAER (μg/min) was calculated by formula: Albumin [mg/dL] x volume of urine in timed collection [dL] x1000)/1440 [min].

At the end, rats were sacrificed and the kidneys were removed, washed with ice-cold saline, weighed and processed for tissue biochemical estimations.

2.4. Estimation of biomarkers of oxidative stress

The dissected kidney was placed in a petri plate with ice-cold conditions. The tissues were sliced using a surgical scalpel in the presence of chilled 0.25 M sucrose. It was then blotted quickly using filter paper. They were minced and homogenized with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 10,000 × g at 0°C using the Remi cooling centrifuge. Either normal saline or phosphate-buffered saline (PBS) was used as the homogenization medium to prepare a 10% w/v tissue homogenate as per the protocol supplied with the respective antioxidant enzyme assay kit. The total protein concentration in the homogenate was determined using a total protein assay kit (Bicinchonic acid method, E-BC-K075).

2.5. Statistical analysis

All the data are expressed as mean ± standard error of the mean (S. E. M.). One-way analysis of variance (ANOVA) was used to record inter-group variation followed by Tukey's multiple comparisons test as appropriate to test statistical significance using GraphPad Prism version 5, Graph Pad Software, Inc. The minimum significance level was set at $p<0.05$ for all tests.

3. Results

3.1. Effect on body weight% and Kidney to body weight ratio

The analysis of the data revealed that the body weights of the diabetic rats was significantly decreased ($p<0.05$) as compared to the control rats at 12^{th} week after the diabetes induction (Table 1). Remarkably, differently treated diabetic rats (DN+CoQ10, DN+NAC, DN+CoQ10+NAC) had significantly restored body weight % (97.62, 94.83, and 108.8%, respectively) after the same period of diabetic induction in comparison to the non-treated diabetic rats. Rats treated by combination of CoQ10 with NAC showed significant ($p<0.05$) restoration of the body weight % to that of the control rats (145.2%), revealing that the treatment by CoQ10 in combination with NAC was better than either that of CoQ10 or NAC only.

ALX treated diabetic rats showed a significant ($p<0.05$) increase in the kidney to body weight ratio (7.64±0.075) as compared to rats in the control group (3.80±0.041). Administration of CoQ10 (10 mg/kg, p.o.), NAC (300 mg/kg, p.o) and the combination of CoQ10 and NAC after induction of DN to the diabetic rats for eight weeks prevented kidney hypertrophy significantly ($p<0.05$) (Table 1).

3.2. Effect on blood glucose level and HbA1c

As per the results presented in Table 2, the blood glucose level of ALX diabetic group was significantly increased to 409.3 mg/dL in comparison to that in the control rats (91.8 mg/dL). Treatment of diabetic rats with CoQ10, NAC, or the combination of CoQ10+NAC for 8 weeks was found to significantly attenuate ($P<0.05$) this elevation of blood glucose level in comparison to the diabetic rats without any treatment. Improvements in the blood glucose level in the three treatment groups (DN +CoQ10, DN + NAC, DN + CoQ10 +NAC) was time-dependent, with the combined treatment of diabetic rats having superior effect in comparison to the other two treated groups with either of the antioxidants alone.

The concomitant administration of CoQ10 and NAC to the diabetic animals also showed beneficial effects in improving HbA1c as shown in Table 2.

3.3. Effect on biochemical parameters

3.3.1. Renal function tests

As shown in Table 3, there was impairment in renal function of diabetic rats as indicated by the levels of serum creatinine, total protein and albumin in comparison to that of control rats. In the ALX-DN rats, a significant increase ($p<0.05$) in the serum creatinine and a significant decrease ($p<0.05$) in protein level was observed. Diabetic rats treated with CoQ10, NAC, or both showed significant ($p<0.05$) improvements in the levels of these biochemical parameters in comparison to that of control diabetic rats. The analysis of results showed that the DN + CoQ10 + NAC rats had superior effect, in returning these parameters towards the control levels.

3.4. Effect on urine volume, creatinine clearance and urinary albumin excretion rate

As shown in Figure 1A, diabetic rats showed a significant increase ($p<0.05$) in 24 h urine volume (mL) which was observed at 95.5±5.15 mL/day in comparison to the control rats with 26.5±1.45mL/day. However, with the improvement of glucose levels in diabetic rats, especially DN +CoQ10 + NAC rats, the urine volume was significantly decreased ($p<0.05$) (38.0±2.12 mL/day) in comparison to the control diabetic rats.

Administration of CoQ10 (10 mg/kg/day, p.o.), NAC (300 mg/kg, p.o.) or their combination (CoQ10+NAC) in diabetic ALX treated rats from fifth to twelfth week of the study significantly ($p<0.05$) improved creatinine clearance and enhanced renal function as compared to the STZ treated diabetic rats. On the other hand, the combined

Table 1: Effect of treatment of CoQ10, NAC or combination of both on body weight% and kidney to body weight ratio in alloxan-induced diabetic rats

Groups	Body weight %		Kidney to body weight ratio (mg/g)
	0 Week	12 Week	
Control	100.0± 0.368	145.2± 0.865$^{\bullet}$	3.807± 0.041
CoQ10(10 mg/kg)	100.0±0.503	137.8±0.712$^{\bullet}$	3.68±0.013
NAC (300 mg/kg)	100.0±1.042	141.3±1.259$^{\bullet}$	3.783±0.045
CoQ10+NAC	100.0±0.574	134.7±1.035$^{\bullet}$	3.712±0.042
ALX- DN	100.0±0.811	85.97±0.748$^{a\bullet}$	7.641±0.075^{a}
DN+CoQ10(10 mg/kg)	100.0±0.833	97.62±0.316^{ab*}	5.304±0.040^{ab*}
DN+ NAC (300 mg/kg)	100.0±0.770	94.83±0.761$^{ab*\bullet}$	5.819±0.099^{ab*}
DN+ CoQ10+NAC	100.0±0.917	108.8±0.914$^{abcd*\bullet}$	4.680±0.039^{abcd*}

Values are expressed as mean±SEM; n=6, evaluated using One way ANOVA followed by Tukey's multiple comparison test. ^{a}p<0.05 as compared to normal control group, ^{b} p<0.05 as compared to diabetic control group, ^{c} p<0.05 as compared to diabetic CoQ10 alone treated group and ^{d} p<0.05 as compared to diabetic NAC alone treated group, $^{\bullet}$ p<0.05 compared with the same group at 0 week and *p< 0.05 vs. the corresponding non-diabetic control group at the corresponding time.

Table 2: Effect of CoQ10, NAC or combination treatment on blood glucose level and HbA1c% in alloxan-induced diabetic rats

Groups	Blood glucose (mg/dL)		HbA1c %
	0 Week	12 Week	
Control	91.83± 2.937	91.0± 3.13	4.733±0.084
CoQ10(10 mg/kg)	84.0±1.612	86.0±2.206	4.567±0.071
NAC (300 mg/kg)	83.17±2.982	86.17±3.114	4.583±0.079
CoQ10+NAC	80.5±2.460	81.33±2.963	4.467±0.088
ALX- DN	409.3±7.369 ^{a}	453.8±8.761$^{a\bullet}$	9.13±0.130^{a}
DN+CoQ10(10 mg/kg)	408.8±8.242 ^{a*}	350.5±11.76$^{ab*\bullet}$	7.05±0.154^{ab*}
DN+ NAC (300 mg/kg)	410.8±7.876 ^{a*}	387.5±9.19^{ab*}	7.15±0.092^{ab*}
DN+ CoQ10+NAC	407.5±7.008^{a*}	315.3±5.56$^{abcd*\bullet}$	5.66±0.315^{bcd}

Values are expressed as mean±SEM; n=6, evaluated using One way ANOVA followed by Tukey's multiple comparison test. ^{a} p<0.05 as compared to normal control group, ^{b} p<0.05 as compared to diabetic control group, ^{c} p<0.05 as compared to diabetic CoQ10 alone treated group and ^{d}p<0.05 as compared to diabetic NAC alone treated group, $^{\bullet}$ p<0.05compared with the same group at 0 week and *p< 0.05 vs. the corresponding non-diabetic control group at the corresponding time.

Table 3: Effect of treatment of CoQ10, NAC or combination of both on body weight% and kidney to body weight ratio in alloxan-induced diabetic rats

Groups	Serum Creatinine (mg/dL)		Total Protein (mg/dL)	Albumin (mg/dL)
	0 Week	12 Week		
Control	0.423±0.044	0.426±0.032	7.55±0.07	3.65±0.04
CoQ10(10 mg/kg)	0.473±0.008	0.453±0.012	7.46±0.07	3.58±0.07
NAC (300 mg/kg)	0.500±0.034	0.520±0.025	7.38±0.09	3.56±0.08
CoQ10+NAC	0.456±0.025	0.473±0.019	7.53±0.10	3.63±0.06
ALX- DN	0.743±0.036^{a}	2.85±0.117$^{a\bullet}$	4.00±0.20^{a}	2.15±0.10^{a}
DN+CoQ10(10 mg/kg)	0.731±0.041^{a*}	1.30±0.085$^{ab*\bullet}$	5.70±0.12^{ab*}	2.98±0.07^{ab*}
DN+ NAC (300 mg/kg)	0.735±0.032^{a*}	1.35±0.061$^{ab*\bullet}$	5.16±0.12^{ab*}	2.93±0.06^{ab*}
DN+ CoQ10+NAC	0.745±0.019^{a*}	0.896±0.035$^{abcd*\bullet}$	6.65±0.15^{abcd*}	3.33±0.10^{bd}

Values are expressed as mean±SEM; n=6, evaluated using One way ANOVA followed by Tukey's multiple comparison test. ^{a} p<0.05 as compared to normal control group, ^{b} p<0.05 as compared to diabetic control group, ^{c} p<0.05 as compared to diabetic CoQ10 alone treated group and ^{d} p<0.05 as compared to diabetic NAC alone treated group, $^{\bullet}$ p<0.05compared with the same group at 0 week and *p< 0.05 vs. the corresponding non-diabetic control group at the corresponding time

administration of CoQ10 and NAC significantly ($p<0.05$) prevented the renal impairment as indicated by improved creatinine clearance as diabetic rats receiving either CoQ10 or NAC alone (Figure 1 B).

UAER calculated at the end of the treatment schedule revealed no significant differences among control and non-diabetic treatment groups as shown in Figure 1 C. Diabetic rats with or without any treatment showed significant ($p<0.05$) rise in UAER in comparison to the control group except the animals received CoQ10+NAC combination. Also, it was observed that, treatment of diabetic rats initiated after completion of fourth week with antioxidants used in this study either alone or in combination significantly ($p<0.05$) reduced UAER as compared to the ALX-DN group.

3.5. Effect on renal oxidative stress

Induction of diabetes significantly ($p<0.05$) impaired oxidative stability because MDA, the end product of lipid peroxidation and marker of oxidative stress levels were found to substantially increased (22.58±0.71 nmol/mg protein) as compared to that of control rats (4.66±0.30 nmol/mg protein). Treatment of diabetic rats with CoQ10, NAC, or both considerably reduced the levels of MDA to the control values, especially in DN+CoQ10 +NAC rats (9.08±0.42 nmol/mg), in comparison to that of control rats. (Figure 2).

3.6. Effect on renal antioxidant status

SOD activity in renal homogenate (Figure 3A) was found unchanged among non-diabetic rats with or without any treatment. Rats rendered diabetic by single i.p. injection of ALX showed significant ($p<0.05$) reduction in SOD activity at end of twelfth week as compared to the control rats without any treatment indicating excessive OS in renal milieu. ALX treated rats administered with CoQ10 or NAC for eight weeks significantly ($p<0.05$) restored SOD activity as compared to the ALX diabetic rats. Concomitant treatment of diabetic rats with CoQ10+NAC showed significant ($p<0.05$) change in SOD activity than diabetic rats received CoQ10 or NAC alone representing beneficial effect of the combination treatment over monotherapy.

As indicated in Figure 3B, significant ($p<0.05$) elevation in CAT activity was observed in the non-diabetic rats received CoQ10, NAC or their combination initiated at the beginning of fifth week and continued for next eight weeks when compared with the control rats without any treatment. The CAT activity was significantly ($p<0.05$) decreased in ALX treated rats, as compared to normal control rats showing the state of excessive OS in this group. CoQ10 or NAC treated diabetic group showed significant increase ($p<0.05$) in the CAT activity when compared to untreated diabetic rats. Moreover, animals in the group DN+CoQ10+NAC showed significant rise ($p<0.05$) in the CAT activity in comparison to the ALX-DN group and diabetic rats receiving CoQ10 or NAC alone.

The endogenous antioxidant GSH levels of diabetic control rats were found to significantly decreased ($p<0.05$) as compared to control values. Treatment of diabetic rats (DN +CoQ10, DN +NAC and DN + CoQ10 +NAC) significantly ($p<0.05$) improved the levels of the GSH in renal homogenate as compared to the diabetic control values (Figure 3C).

4. Discussion

The aim of the present investigation was to determine the effect of CoQ10 and NAC alone and in combination to reduce nephropathy in ALX-induced diabetic rat model. ALX is a frequently used diabetogenic agent for inducing T1DM and consequently, type 1DN (T1DN). T1DN develops within 3-4 weeks after injection of ALX.[12] The diabetogenic effects of ALX resulting in necrosis of β-cells are mediated via chronic hyperglycemia and subsequent production of ROS culminates in diabetic renal damage and T1DN.[13,14]

In the present study, single injection of ALX (130 mg/kg, i.p.) resulted in hyperglycemia in rats which further resulted in renal dysfunction as shown by increased serum creatinine, decreased total protein and albumin with declined creatinine clearance and increased UAER in comparison to the control rats. On the other hand, treatment of DN rats with CoQ10 and NAC significantly restored kidney function and ameliorated OS.

In the current investigation, a significant reduction in body weight % in diabetic rats was recorded in ALX-induced DN. This decrease in the body weight may be related to the degradation of structural proteins contributing to body weight. It was showed that body weight loss occurred as a result of increased muscle wasting owing to loss of tissue proteins.[15]

The results of the present study demonstrated that diabetic rats treated with CoQ10, NAC or their combination improved body weight % significantly as indicated by healthier appearance of animals in the DN groups receiving these treatments. CoQ10 shown to contribute in insulin cascade and improve GLUT2 function leading to enhanced glucose uptake and inhibition of gluconeogenesis.[16] It was reported that NAC improves aerobic glucose metabolism and glycogenesis in addition to the reducing insulin resistance.[17] Thus, inhibition of weight loss and improved growth and health status of the diabetic animals might be attributed to the protective effect of these antioxidants in controlling muscle wasting and loss of tissue proteins by inhibiting gluconeogenesis, increasing glycogenesis and reducing insulin resistance.

The kidney to body weight ratio of the diabetic rats was increased significantly in the ALX induced DN model

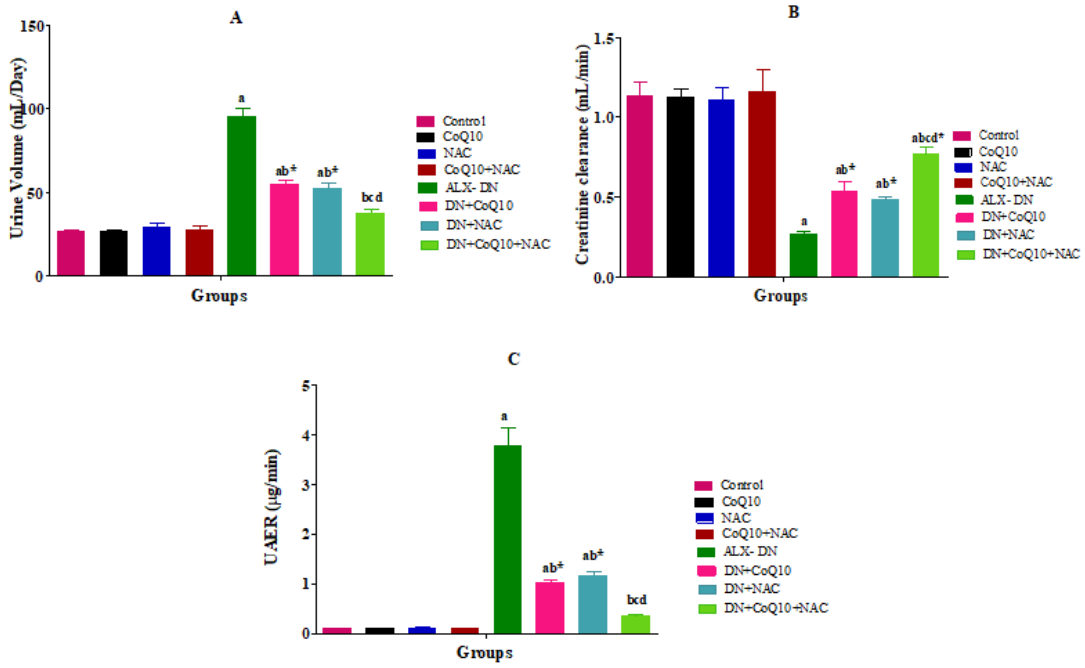

Fig. 1: Effects of CoQ10, NAC or combination of both on **A:** urine volume; **B:** Creatinine clearance and **C:** UAER in ALX-induced diabetic rats Values are expressed as mean±SEM; n=6, evaluated using One way ANOVA followed by Tukey's multiple comparison test. ^{a}p<0.05 as compared to normal control group, ^{b}p<0.05 as compared to diabetic control group, ^{c}p<0.05 as compared to diabetic CoQ10 alone treated group and ^{d}p<0.05 as compared to diabetic NAC alone treated group, *p< 0.05 vs. the corresponding non-diabetic control group at the corresponding time.

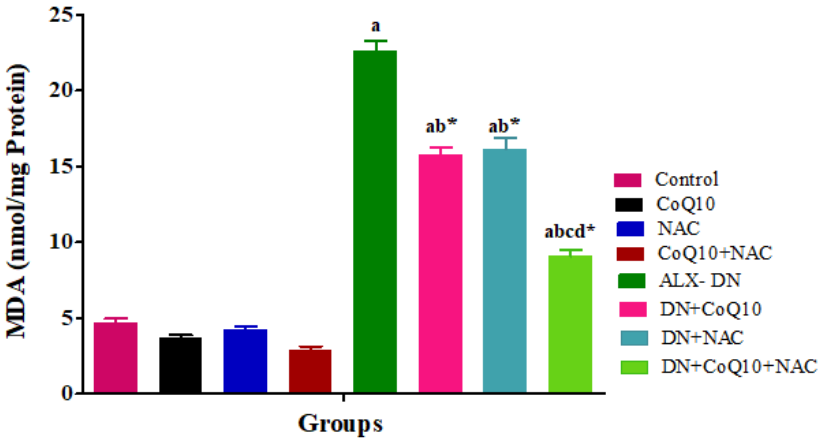

Fig. 2: Effects of CoQ10, NAC or combination of both on MDA in ALX-induced diabetic rats Values are expressed as mean±SEM; n=6, evaluated using One way ANOVA followed by Tukey's multiple comparison test. ^{a}p<0.05 as compared to normal control group, ^{b}p<0.05 as compared to diabetic control group, ^{c}p<0.05 as compared to diabetic CoQ10 alone treated group and ^{d}p<0.05 as compared to diabetic NAC alone treated group, *p< 0.05 vs. the corresponding non-diabetic control group at the corresponding time.

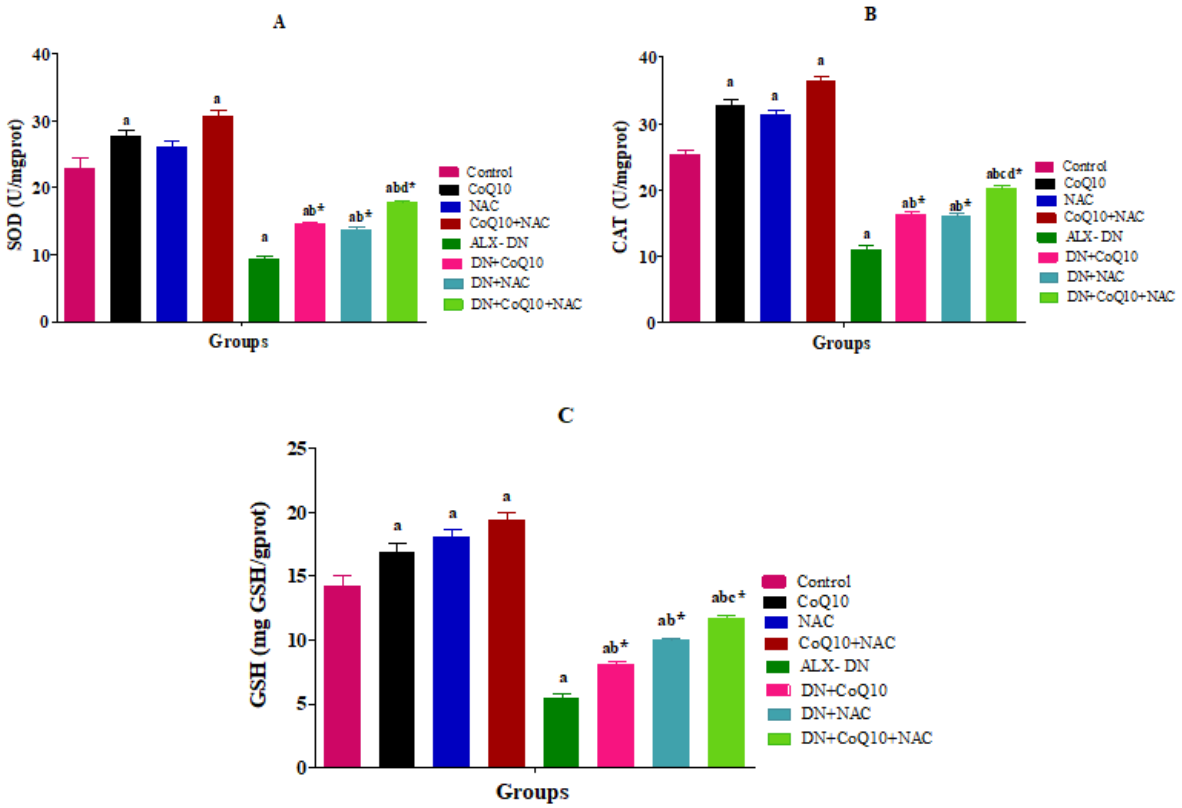

Fig. 3: Effects of CoQ10, NAC or combination of both on **A:** SOD, **B:** CAT and **C:** GSH in ALX-induced diabetic rats Values are expressed as mean±SEM; n=6, evaluated using One way ANOVA followed by Tukey's multiple comparison test. ^{a}p<0.05 as compared to normal control group, ^{b}p<0.05 as compared to diabetic control group, ^{c}p<0.05 as compared to diabetic CoQ10 alone treated group and ^{d}p<0.05 as compared to diabetic NAC alone treated group, *p< 0.05 vs. the corresponding non-diabetic control group at the corresponding time.

suggesting the renal hypertrophy, the key feature in initial alteration by DM. These findings were consistent to the previous studies with experimentally induced diabetes in animals.[18,19] It has been described that kidney hypertrophy leads to increased kidney weight in DM due to glucose over-utilization, glycogen accumulation, lipogenesis and protein synthesis in the renal tissue.[20] In the results presented, treatment with CoQ10 and /or NAC significantly decreased the renal hypertrophy in diabetic rats. This suggests the potential of CoQ10 and NAC in attenuating renal injury and imparting renal protection. This protective action of CoQ10 and/or NAC may be related to inhibition of compensatory renal growth attributed to above mentioned mechanisms in reducing renal hypertrophy. Thus, CoQ10, NAC or their combination treatment demonstrated reversal of kidney hypertrophy in DN rats.

Persistent hyperglycemia is the key factor in structural alterations at the renal level, and; therefore, glycemic control remains the main target of therapy of DN. In this study, the blood glucose and HbA1c level of rats with DN showed significant increase; however, treatment of DN animals with CoQ10, NAC, or their combination restricted this increase in blood glucose level.

Studies reported the deficiency of CoQ10 in diabetic individuals which may impair the body's defensive mechanisms against hyperglycemia induced OS during DM.[21–23] Several reports explained the effect of CoQ10 on glycemic control. Mezawa et al showed that the reduced form of CoQ10 i.e. ubiquinol, increases insulin production and/or secretion by triggering mitochondrial ATP production in pancreatic β-cells.[23] Thus, exogenously administered CoQ10 could potentially attenuate mitochondrial dysfunction induced by OS, thus improving glycemic control in DM.[24] Several studies reported the efficacy of NAC against insulin resistance and associated complications. In most of these studies, NAC reduced hyperglycemia due to improvement in the insulin sensitivity and enhancing peripheral glucose uptake by its antioxidant propertie.[17]

Thus, the combined treatment employed herein may have a synergistic effect of CoQ10 and NAC in reducing hyperglycemia and HbA1c.

Elevated levels of serum creatinine are associated with interstitial atrophy, epithelial necrosis as well as atrophic changes in the glomeruli and thus DN.[25] In this study, diabetic rats have shown a significant increase in serum creatinine with decline in the creatinine clearance, but 8 weeks of CoQ10 and NAC treatment significantly reduces these levels indicating its protective role in halting the progression of DN.

Increased in UAER is an indicator of pathophysiological alterations in the kidney such as lesions in glomerular basement membrane and key to diagnose nephropathy in diabetic patients.[26] Chronic hyperglycemia in diabetic rats results in increased UAER indicating the progression of DN, whereas, 8 weeks of CoQ10 and NAC treatment significantly reduced UAER, thus exerting ameliorative effect on diabetic kidneys.

OS plays an important role in the pathogenesis of DN through overproduction of active carbonyl intermediates, reduction of antioxidant enzyme activities, formation of lipid peroxides and ROS. Antioxidant enzymes prevent injuries at cellular and tissue levels. An imbalance between ROS production and antioxidants is the key element in DN.[27] In the present study, induction of Type 1 DM with ALX caused a significant elevation of OS as indicated by increased MDA and decreased activities of the antioxidant enzymes SOD and CAT along with decline in the endogenous antioxidant GSH. This could be attributed to the pathogenesis in the rat kidney and the progression of DN. Treatment of rats with CoQ10 and/or NAC for 8 weeks post DN confirmation reversed these abnormalities, which is consistent with previous work of Maheshwari et al. and Lee et al. and others.[8,10]

Thus, the oral administration of CoQ10 or NAC markedly reduced the imbalance between ROS and restored antioxidant enzymes activity confirms the antioxidant potential of these agents in DN. Therefore, antioxidants like CoQ10 and NAC are useful in prevention and improvement of deteriorating renal function during the course of DN.

5. Conclusion

the results of the present study demonstrated that combination treatment with CoQ10 and NAC associated with a synergetic inhibition of various markers in renal tissue of DN induced rats which indicate the renoprotective effect of combined therapy. The study provides the evidence supported concept that hyperglycemia induced OS is implicated in the development and progression of renal injury through induction of various pathways which further induces ROS production and aggravates DN. Therefore, administration of CoQ10 and NAC in combination targeting diverse pathogenic mechanisms led to the prevention and progression of DN through attenuation of induced OS and restoration of antioxidative enzymes.

6. Source of Funding

None.

7. Conflict of Interest

None.

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