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ORIGINAL ARTICLE

Elucidation of molecular mechanism involved in neuroprotective effect of Coenzyme Q10 in alcohol-induced neuropathic pain

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ABSTRACT

The aim of the present investigation was to evaluate the effect of Coenzyme Q10 and its combination with vitamin E in alcohol-induced chronic neuropathic pain. Male Wistar rats were orally treated with alcohol (10 g/kg, 35% v/v, b.i.d.) for 10 weeks. Coenzyme Q10 (25, 50, and 100 mg/kg) and vitamin E (100 mg/kg) were coadministered orally for 1 h after ethanol administration for 10 weeks. Various nerve functions, biochemical, and molecular parameters were assessed. Chronic administration of ethanol for 10 weeks resulted significant development of neuropathic pain. Treatment with Coenzyme Q10 (50 and 100 mg/kg) for 10 weeks showed significant and dose dependently increased in level of nociceptive threshold, endogenous antioxidant, and Na,K-ATPase enzyme. Coenzyme Q10 (50 and 100 mg/kg) significantly restored the levels of motor nerve conduction velocity and sensory nerve conduction velocity. It also showed significant decrease in levels of endogenous calcium, oxidative–nitrosative stress, TNF-α, IL-1β, and IL-4 level. Alteration in protein expression of polymerase gamma (pol γ) was significantly restored the Coenzyme Q10 treatment. The important finding of the study is that, Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination-treated rats showed more significant prevention of behavioral, biochemical, and molecular neurotoxic effect of alcohol administration than Coenzyme Q10 or α-tocopherol alone treated group. It is evident from the finding of present investigation that plethora of mechanism including inhibition of oxido-nitrosative stress, release of pro-inflammatory cytokine, modulation of endogenous biomarker, and protection of pol γ protein expression simultaneously orchestrate to exhibits neuroprotective effect of Coenzyme Q10, vitamin E and their combination.

INTRODUCTION

Chronic alcohol consumption leads to an array of pathological transformations leading to peripheral polyneuropathy, Wernicke encephalopathy, cortical, and motility dysfunction, psychosis and delirium tremens and is a burring major social abuse [1]. Around 10–50% of the population of the world is suffering from peripheral neuropathy [2].

From the ancient times, alcohol-induced central nervous system disorders have been limelight, as its chronic administration causes disturbances of psychic and motor functions. Alcohol exerts its catastrophic effect on all the organ system by means of a small-fiber

dying-back mechanism [3,4]. Administration of ethanol in rodent precipitated neurotoxicity due to imbalance in membrane lipid composition and fluidity [5]. Malnutrition, environmental, and psychological factors leads to motor dysfunction in humans.

In rodents, a variety of pathological changes are apparent and various facets of peripheral painful neuropathy are exhibited. Alcohol-induced neuropathic pain is well established, reproducible laboratory animal model for preclinical evaluation of potential drug for neuropathy pain induced by chronic alcohol consumption [6,7]. Hyperalgesia, allodynia, alleviation of oxidative stress, reduction of nerve conduction velocity, surge of cytokines, overexpression of inflammatory mediators, DNA damage, imbalance between OXPHOS pathway, disregulated energy metabolism affects neural fibers and axon incapacitating antioxidant and electron transfer homeostasis [8,9]. Chronic ethanol consumption causes decreasing endogenous antioxidants (α tocopherol, ascorbate, and vitamin E) concentration, which resulted generation of reactive oxygen species and lipid peroxidation [10]. Hence, endogenous antioxidants have evolved as attractive target holding promise to reverse alcohol-induced neuropathic dysfunction.

An array of clinical trials has been conducted to determine the effect of various synthetic chemical moieties in alcohol-induced neuropathic pain [11]. The current treatment regimen of peripheral neuropathy includes gabapentin, amitriptyline, vitamins B_1 (thiamine), B_2 (riboflavin), B_6 (pyridoxine), B_9 (folic acid), and B_{12} (cyanocobalamin) along with OTC pain medications like aspirin or acetaminophen [12]. However, promising outcomes have not been observed.

Coenzyme Q10 or ubiquinone or 2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone is a liquid-soluble potent antioxidant and anti-inflammatory substance akin to vitamin [13]. It occurs abundantly in dietary items including meat, poultry, fish, vegetable oils, and nuts [14]. Coenzyme Q10 is a vital cofactor in complexes I to III of the mitochondrial electron transport chain, which serves as either as an electron acceptor or donor [15]. Due to its structural characteristic ability to modify and get diffuse into the membrane phospholipid bilayer, which repair and enhance disregulated ATP synthesis in the mitochondria, it serves as powerful antioxidant [16]. The previous studies carried out by Kohli et al. [17] and El-Abhar [18] shown that Coenzyme Q10 possesses anti-ulcer potential. It's a well-known radical scavenger, [19] which protects cells from oxidative stress in vivo [20]. Extensive research on Coenzyme Q10 shown that it has anti-inflammatory as well as anti-diabetic properties [21,22]. Previous studies demonstrated that coenzyme Q10 effectively protected against testicular injury induced by sodium arsenite, magnetic field exposure, and ischemia/reperfusion in laboratory animals [23–25]. Ghule et al. have documented that Coenzyme Q10 provides protection against isoproterenol-induced cardiotoxicity and cardiac hypertrophy preclinically, and Burke et al. shown that Coenzyme Q10 may be the treatment for systolic hypertensive patient [26,27].

Hence, the present investigation was designed to evaluate the effect of Coenzyme Q10 and its combination with vitamin E in alcohol-induced chronic neuropathic pain by assessing various nerve functions, biochemical parameters, molecular parameters as well as DNA fragmentation.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (150–200 g) were obtained from the National Institute of Biosciences, Pune (India). They were maintained at 24 ± 1 °C, with relative humidity of 45–55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro industries Ltd., Sangli, India) and water throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune (CPCSEA/74/2010) and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

Drugs and chemicals

Coenzyme Q10 was supplied by Medicines Pvt. Ltd. (Mumbai, India), and α-tocopherol was purchased from Sigma Chemical Co. (St Louis, MO, USA). 1,1′,3,3′-Tetraethoxypropane, crystalline beef liver catalase, reduced glutathione (GSH), 5,5′-dithiobis (2-nitrobenzoic acid), bovine serum albumin, thiobarbituric acid, Tris buffer, sucrose, trichloroacetic acid, citric acid monohydrate, sodium nitrate, copper sulfate, sodium potassium tartarate, ethylene diamine tetra acetic acid disodium salt, Folin's phenol reagent, sodium hydroxide, sodium carbonate, magnesium chloride, sodium carbonate, sodium bicarbonate, potassium chloride, calcium chloride, disodium hydrogen orthophosphate, potassium

dihydrogen orthophosphate, carbon tetrachloride, chloroform, ether, hydrochloric acid, and conc. sulfuric acid were purchased from S.D. Fine Chemicals, Mumbai, India. Sulphanilamides, naphthalamine diamine HCl, and phosphoric acid were obtained from LobaChemi Pvt. Ltd., Mumbai, India. TNF-α and IL-1β ELISA kit were obtained from Thermo Scientific, San Jose, CA, USA. IL-4 ELISA kit was obtained from RayBiotech, Inc. Norcross, GA, USA.

Preparation of drug solutions

Coenzyme Q10 was freshly prepared in 1% aqueous solution of Tween 80 in three different dosages (20, 50, and 100 mg/kg) and administered to rats orally for 10 weeks [26,28]. α-Tocopherol was freshly prepared in 1% aqueous solution of Tween 80 and administered to rats orally at a dose of 100 mg/kg for 10 weeks [7].

Induction of alcoholic neuropathy and drug treatment schedule

Alcoholic neuropathy was induced by administration of 35% v/v ethanol (10 g/kg, b.i.d. [bis in die, i.e., twice daily], oral gavage) in double distilled water for 10 weeks according to method reported by [6,7].

The 72 animals were divided into following groups, each consisting of eight rats. A suspension of Coenzyme Q10 in three different dosages (25, 50, and 100 mg/kg) was administered to animals orally for 10 weeks.

(A) Animals without ethanol administration:
- Group I – (N): Normal control (10 g/kg of 1% tween 80, p.o. [per os, i.e., by oral route])
- Group II – (CoQ10 (100)) (per se): Coenzyme Q10 (100 mg/kg), p.o.
- Group III – (α-T (100)) (per se): α -tocopherol (100 mg/kg), p.o.

(B) Animals with ethanol administration:
- Group IV – (EC): Ethanol control (10 g/kg of 35%, v/v, ethanol, b.i.d., p.o.)
- Group V – (E + α-T (100)): Ethanol (b.i.d.) + α-tocopherol (100 mg/kg) (1 h before ethanol admi.), p.o.
- Group VI – (E + CoQ10 (25)): Ethanol (b.i.d.) + Coenzyme Q10 (25 mg/kg) (1 h before ethanol admi.), p.o.
- Group VII – (E + CoQ10 (50)): Ethanol (b.i.d.) + Coenzyme Q10 (50 mg/kg) (1 h before ethanol admi.), p.o.
- Group VIII – (E + CoQ10 (100)): Ethanol (b.i.d.) + Coenzyme Q10 (100 mg/kg) (1 h before ethanol admi.), p.o.
- Group IX – (E + C (100) + α-T (100)): Ethanol (b.i.d.) + Coenzyme Q10 (100 mg/kg) + α-tocopherol (100 mg/kg) (1 h before ethanol admi.), p.o.

All the behavioral assays were performed by an observer blind to the drug administration on 1st, 2nd, 4th, 6th, 8th, and 10th week. Food intake and water intake were measured with the help of metabolic cage (Metabolic cage, Techniplast, Italy).

Behavioral tests

Thermal hyperalgesia (radiant heat test)

Radiant heat hyperalgesia of the left hind paw was assessed using the radiant heat lamp source as described method of Hargreaves et al. [29] for assessing the reactivity to noxious thermal stimuli. The intensity of the radiant heat stimulus was maintained at 55 ± 0.1 °C. Response of left hind paw withdrawal threshold was noted. Cut-off time of 10 s was maintained.

Mechanical hyperalgesia (Randall–Selitto paw pressure test)

Mechanical nociceptive threshold, an index of mechano-hyperalgesia, was assessed by method described by Randall and Selitto [30]. The nociceptive flexion reflex was quantified using the Randall–Selitto paw pressure device (UGO Basile SRL Biological Research Apparatus, Comerio, Italy), which applies a linearly increasing mechanical force (g) to the dorsum of the rat's hind paw. The paw of the rat was placed under the tip, and the progressive pressure applied until the rat vocalized. The nociceptive threshold was expressed in grams and measured three or four times to obtain two consecutive values that differed no 10%, respecting an interval of at least 10 min between two measures. The withdrawal of the hind paw was used to assess the nociceptive threshold.

Mechano-tactile allodynia (Von frey hair test)

Mechano-tactile allodynia (non-noxious mechanical stimuli) was assessed as described by Chaplan et al. [31]. Rats were placed individually on an elevated mesh (1 cm^2 perforations) in a clear plastic cage and adapted to the testing environment for at least 15 min. von Frey hairs (IITC, Woodland Hills, CA, USA) with calibrated bending forces (g) of different intensities were used to deliver punctuates mechanical stimuli of varying intensity. von Frey hairs were applied from below the mesh floor to the plantar surface of the hind paw, with sufficient force to cause slight bending against the paw, and held for 1 s. Each stimulation

was applied five times with an interstimulus interval of 4–5 s. Care was taken to stimulate random locations on the plantar surface. A positive response was noted if the paw was robustly and immediately withdrawn. Paw withdrawal threshold was defined as the minimum pressure required to elicit withdrawal reflex of the paw, at least one time on the five trials.

Thermal hyperalgesia (tail immersion test)

Spinal thermal sensitivity was assessed by the tail immersion test as described by Necker and Hellon [32]. In this test, tail of rat was immersed in a water bath maintained at 55 °C until tail withdrawal or signs of struggle were observed (cut-off time 15 s). The reaction time (i.e., the time necessary to observe the withdrawal of the tail from the bath) was measured 2–3 times to obtain two consecutive values that differed no 10%. The tail of the rat was immediately dried with a soft cellulose paper to avoid tail cooling between two measures. A shortened duration of tail withdrawal indicates thermal hyperalgesia.

Motor co-ordination test

Motor incoordination was evaluated by a Rota-Rod device as described by Jones and Roberts [33]. Rats were placed for 1 min on the rotating rod of Rota-Rod apparatus (Techno Rotarod, Lucknow, India). The time taken for the falling of the rat from the rotating rod, during the period of 1 min was recorded.

Motor nerve conduction velocity

The recording of motor nerve conduction velocity (MNCV) was performed in rats according to previously described method Kandhare et al. [34]. Briefly, rats were anesthetized using thiopental sodium (50 mg/kg, i.p.) for electrophysiological recording. The dorsal side of the rats paw was shaved with hair removal cream and cleaned using moist cotton plug. The rats were placed in a ventral position and were warmed on a heated pad to keep the temperature of the tail surface at 33–35 °C [35]. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch respectively by 200 μs square wave pulse delivered through a pair of monopolar needle electrodes (1.0–1.5 mA, 2.0 mV/D) using a stimulator (Weltronics Systems, Pune, India). Responses were recorded from the plantar muscles using data acquisition system (LabChart 7.3; AD Instrument Pvt. Ltd., Bella Vista, Australia). The MNCV was determined using the following formula:

$$\text{MNCV} = [\text{Distance between sciatic and tibial stimulation point (in m)}]/[\text{latency for sciatic (in s)} - \text{latency for tibial (in s)}]$$

Sensory nerve conduction velocity

Sensory nerve conduction velocity (SNCV) was measured by a modified method of Misumi and Cavaletti et al. [36,37]. Briefly, rats were anesthetized using thiopental sodium (50 mg/kg, i.p.) for electrophysiological recording. The stratum corneum at the stimulating and recording sites was shaved with hair removal cream and cleaned using moist cotton plug. The rats were placed in a ventral position and were warmed on a heated pad to keep the temperature of the tail surface at 33–35 °C [35]. Ring electrodes were used to record the nerve potentials. For the first recording point, the cathode was placed approximately 2 cm from the anus, the anode being placed 1 cm proximal to the cathode. A 200 μs square wave pulse delivered through a pair of monopolar needle electrodes (1.0–1.5 mA, 2.0 mV/D) using a stimulator (Weltronics). Responses were recorded from the plantar muscles using data acquisition system (LabChart 7.3; AD Instrument Pvt. Ltd.,). SNCV was calculated by dividing the distance by the difference in latencies at the two recording points.

Biochemical estimations

Sciatic nerve homogenate preparation

All animals were sacrificed at the end of study, that is, 8th week, and sciatic nerves were immediately isolated. Tissue homogenates were prepared with 0.1 M Tris–HCl buffer (pH 7.4), and supernatant of homogenates was employed to estimate superoxide dismutase (SOD), reduced GSH, lipid peroxidation (malondialdehyde [MDA] content), nitric oxide (NO content), total calcium content, membrane-bound inorganic phosphate (Na^+,K^+ATPase), TNF-α, IL-1β, and IL-4.

Determination of total protein

Protein concentration was estimated according to the method of Lowry et al. [38], using bovine serum albumin (BSA) as a standard.

Determination of SOD contents

The neural pathological alteration occurs due to the overproduction of reactive oxygen species (ROS). SOD assay was determined as previously described by Misera and Fridovich [39]. SOD activity was expressed as U/mg of protein.

Determination of GSH contents

The GSH assay was performed according to method previously described by Moron et al. [40]. The amount of reduced GSH was expressed as μg/mg of protein.

Determination of MDA content

Malondialdehyde levels in the neural tissue were determined by the method of Slater and Sawyer [41]. The values were expressed in nanomoles/mg of protein.

Determination of nitrite level

The NO level was estimated as nitrite by the acidic Griess reaction after reduction in nitrate to nitrite by vanadium trichloride according to the method described by Miranda et al. [42]. The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide, and *N*-(1-naphthyl) ethylenediamine to produce a pink azo product with maximum absorbance at 543 nm. The concentrations were determined using a standard curve of sodium nitrate, and the results were expressed in μg/mL.

Determination of total calcium

Total calcium levels were estimated in sciatic nerve as described by Severinghaus and Ferrebee [43]. Briefly, sciatic nerve homogenate was mixed with 1 mL of trichloroacetic acid (4%) in ice-cold conditions and centrifuged at 180 ***g*** for 10 min. The clear supernatant was used for the estimation of total calcium by flame photometry method.

Determination of membrane-bound inorganic phosphate (Na^+,K^+ATPase)

Membrane-bound inorganic phosphate (Na^+,K^+ATPase) was estimated in sciatic nerve as described by Bonting [44]. In brief, 1.0 mL of tris-hydrochloride buffer and 0.2 mL each of magnesium sulfate, sodium chloride, potassium chloride, EDTA, ATP were added to test tube containing 0.2 mL of sciatic nerve homogenate. The mixture was incubated at 36 °C for 15 min. The reaction was arrested by the addition of 1.0 mL of 10% TCA, mixed well, and centrifuged. The enzyme activity was expressed in μM of inorganic phosphorus liberated/mg of protein/min.

Determination of TNF-α and IL-1β

The quantifications of TNF-α and IL-1β were performed with the help and instructions provided by Thermo Scientific. Rat TNF-α and IL-1β immunoassay kit contains rat TNF-α and IL-1β immunoassay, which is a 4.5-h solid-phase ELISA, designed to measure rat TNF-α and IL-1β levels. The assay employs the sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat TNF-α and IL-1β had been precoated in the microplate. Briefly, 50 μL of pretreated buffer was added to each well. Then, 50 μL of standards, control, and test samples (aliquot of sciatic nerve homogenate) were added into each well and incubated at R.T. for 1 h. If any rat TNF-α and IL-1β is present, it would have bound by the immobilized antibody. After having washed away any unbound substance, 50 μL of biotinylated antibody reagent was added to each well and incubated at R.T. for 1 h. After washing away any unbound substance, 100 μL of streptavidin-HPR reagent was added to each well, which is an enzyme-linked polyclonal antibody specific for rat TNF-α. Then, it was followed by washing to remove any unbound antibody–enzyme reagent. The 100 μL of TMB, a substrate solution and consequently an enzyme reaction, was added, which made the blue product to turn yellow. The intensity of the color was measured at 550 nm, in proportion to the amount of rat TNF-α and IL-1β bound in the initial steps. The sample values were then read off using the standard curve. Values were expressed as means ± SEM.

Determination of IL-4

The quantifications of IL-4 were performed with the help and instructions provided by RayBiotech, Inc. Rat IL-4 immunoassay kit contains rat IL-4 immunoassay, which is a 4.5-h solid-phase ELISA designed to measure rat IL-4 levels. The assay employs the sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IL-4 had been precoated in the microplate. Briefly, 100 μL of standards, control, and test samples (aliquot of sciatic nerve homogenate) were added into each well and incubated at R.T. for 2.5 h. If any rat IL-4 is present, it would have bound by the immobilized antibody. After having washed away any unbound substance, 100 μL of biotinylated antibody reagent was added to each well and incubated at R.T. for 1 h. After washing away any unbound substance, 100 μL of streptavidin-HPR reagent was added to each well, which is an enzyme-linked polyclonal antibody specific for rat IL-4 and incubated at R.T. for 45 min. Then, it was followed by washing to remove any unbound antibody-enzyme reagent. The 100 μL of TMB a substrate solution and consequently an enzyme reaction was added, which made the blue product to turn yellow. The intensity of the color was measured

at 450 nm, in proportion to the amount of rat IL-4 bound in the initial steps. The sample values were then read off using the standard curve. Values were expressed as means ± SEM.

Determination of DNA damage by reverse transcriptase PCR

RNA Isolation

The nerve tissue was chopped and minced. The specimens were disrupted using mortar and pestle in liquid nitrogen. Total cytoplasmic RNA was extracted from nerve samples using a guanidium isothiocyanate/phenol chloroform/Trizol method (Thermo Fischer Pvt. Ltd., Mumbai, India). Following an isopropanol precipitation, the RNA was washed with 70% ethanol and treated with RNase Inhibitor (Thermo Fischer) for 45 min. Following resuspension of the RNA at 65 °C for 15 min, RNA preparations were further purified using the Qiagen RNA isolation kit and were treated with RNase-free DNase as directed by the manufacturer (Qiagen, Valencia, CA, USA). Following precipitation, RNA was resuspended in RNase-free water, and its concentration was quantified by absorbance at 260-nm wavelength. RNA samples were stored at −80 °C until analysis.

cDNA Preparation

Single-stranded cDNA was synthesized from 5 μg of total cellular RNA using reverse transcriptase (Takara, Mountain View, CA, USA) and oligo-(dT)-primers (Takara) as described previously [45]. Briefly, 5 μg of total RNA was uncoiled by heating (65 °C for 5 min), and then reverse transcribed into complementary DNA (cDNA) in a 50-μL reaction mixture that contained 50 U Moloney murine leukemia virus reverse transcriptase (MMLV-RT), 0.3 μg oligo-(dT)-primer, 1 μL RNase Block Ribonuclease Inhibitor (40 U/μL), 2 μL of a 100-mM mixture of deoxyadenosinetriphosphate (dATP), deoxyribothymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP), and deoxycytidine triphosphate (dCTP), 5 μL 10× RT buffer (10 mM Tris–HCl, pH = 8.3, 50 mM KCl, 5 mM $MgCl_2$). The resultant cDNA (2 μL) was amplified in a 50-μL reaction volume containing 2 U Taq polymerase, 200 μM (each) dNTP (Vivantis, Oceanside, CA, USA), 1.5 mM $MgCl_2$, 5 μL 10× polymerase chain reaction buffer (50 nM KCl, 10 nM Tris–HCl, pH = 8.3) and specific primers used at final concentration of 0.5 μM.

The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Eppendorf India Ltd, Chennai, India). The primer sequence for polymerase gamma (pol γ) and β-actin were based on the basis methodology of Lin et al. [46] and is in *Table I*. Amplification of β-actin served as a control for sample loading and integrity. The primers were synthesized by Ocimum Biosolutions, Hyderabad. Polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Size of amplicons was confirmed by using 100-bp ladder (Takara) as a standard size marker. The amplicons was visualized, and image was captured using gel documentation system (Alpha Innotech Inc., San Leandro, CA, USA).

Table I Primer sequences for polymerase gamma (pol γ) and β-actin.

Gene	Primer sequences (5′–3′)	Annealing temperature (°C)	Length (bp)
pol γ	ATGTCCTTGTGGGTGCTG GCGGCTGTCTTACTGTGC	58	162
β-actin	GACAGGATGCAGAAGGAGATTACT TGATCCACATCTGCTGGAAGGT	58	142

The expression of all the genes was assessed by generating densitometry data for band intensities in different sets of experiments and was generated by analyzing the gel images on the Image J program (Version 1.33, Wayne Rasband, National Institutes of Health (NIH), Bethesda, MD, USA) semiquantitatively. The band intensities were compared with constitutively expressed β-actin. The intensity of mRNAs was standardized against that of the β-actin mRNA from each sample, and the results were expressed as PCR-product/β-actin mRNA ratio.

Statistical analysis

Data were expressed as mean ± standard error mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data of behavioral tests were statistically analyzed using two-way repeated analysis of variance (ANOVA), and Bonferroni's multiple range test was applied for post hoc analysis, while data of biochemical parameters were analyzed using one-way analysis of variance (ANOVA), and Tukey's multiple range test was applied for post hoc analysis. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of Coenzyme Q10 on body weight, water intake, and food intake

Body weight, water intake, and food intake in ethanol control rats (200.80 ± 7.88 g, 45.20 ± 1.15 mL, and

24.52 ± 0.73 g, respectively) did not differ significantly than that in the normal rats on 0th week (196.80 ± 4.21 g, 43.96 ± 1.44 mL, and 26.02 ± 1.22 g, respectively). The body weight of the normal rats on 10th week was 278.80 ± 4.35 g, whereas the water intake and food intake were 55.20 ± 1.54 mL and 32.46 ± 1.74 g. Chronic ethanol administration (10 g/kg, 35% v/v) for 10 weeks significantly ($P < 0.05$) decreased body weight (215.50 ± 7.14 g), water intake (35.22 ± 1.12 mL), and food intake (16.24 ± 1.25 g) in ethanol control rats as compared with normal rats. In rats receiving concomitant with Coenzyme Q10 (25, 50 and 100 mg/kg) for 10 weeks showed significant and dose-dependent inhibition ($P < 0.05$) of this reduction in the body weight (217.80 ± 5.41, 236.80 ± 3.54, and 247.60 ± 4.27 g), water intake (37.84 ± 1.36, 41.98 ± 0.98, and 46.30 ± 1.48 mL) and food intake (18.60 ± 1.04, 23.22 ± 0.77, and 26.28 ± 0.86 g) as compared with ethanol control rats. When compared with ethanol control rats, α-tocopherol (100 mg/kg)-treated rats showed significant increased ($P < 0.05$) in this body weight (256.20 ± 5.78 g), water intake (49.28 ± 0.97 mL), and food intake (29.12 ± 0.98 g). Moreover, the attenuation of ethanol-induced decreased body weight, water intake, and food intake by Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination-treated group was more significant (271.40 ± 2.83, 51.20 ± 0.82, and 31.08 ± 0.74 g, respectively, $P < 0.05$) than Coenzyme Q10 or α-tocopherol alone treated groups. There was no significant change in the body weight, water intake, and food intake in per se group over the same time period (*Figure 1*).

Effect of Coenzyme Q10 on thermal hyperalgesia (Hargreaves test)

There was no significant difference in the mean paw withdrawal latency in ethanol-treated rats (8.21 ± 0.29 s) as compared with normal rats (7.91 ± 0.29 s) before administration of ethanol (10 g/kg, 35% v/v). Ethanol administration for 10 weeks significantly decreased ($P < 0.05$) mean paw withdrawal latency in ethanol

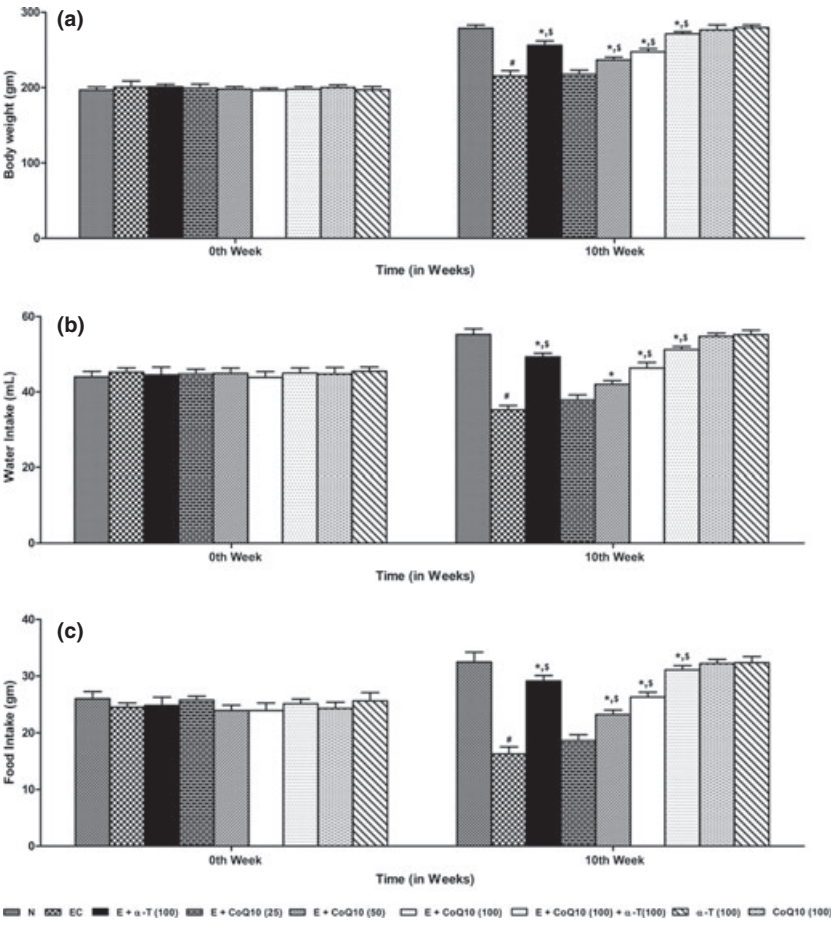

Figure 1 Effect of Coenzyme Q10 and α-tocopherol treatment on body weight (a), water intake (b) and food intake (c) in ethanol treated rats. Data are expressed as mean ± SEM ($n = 6$) and analyzed by two way ANOVA followed by Bonferroni's test. *$P < 0.05$ as compared to the ethanol control group, #$P < 0.05$ as compared to normal group and $$P < 0.05$ as compared to one another. N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg) treated rats; E + CoQ10 (25), ethanol + Coenzyme Q10 (25 mg/kg) treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg) treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg) treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination treated rats; α-T (100), α-tocopherol (100 mg/kg) alone treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats.

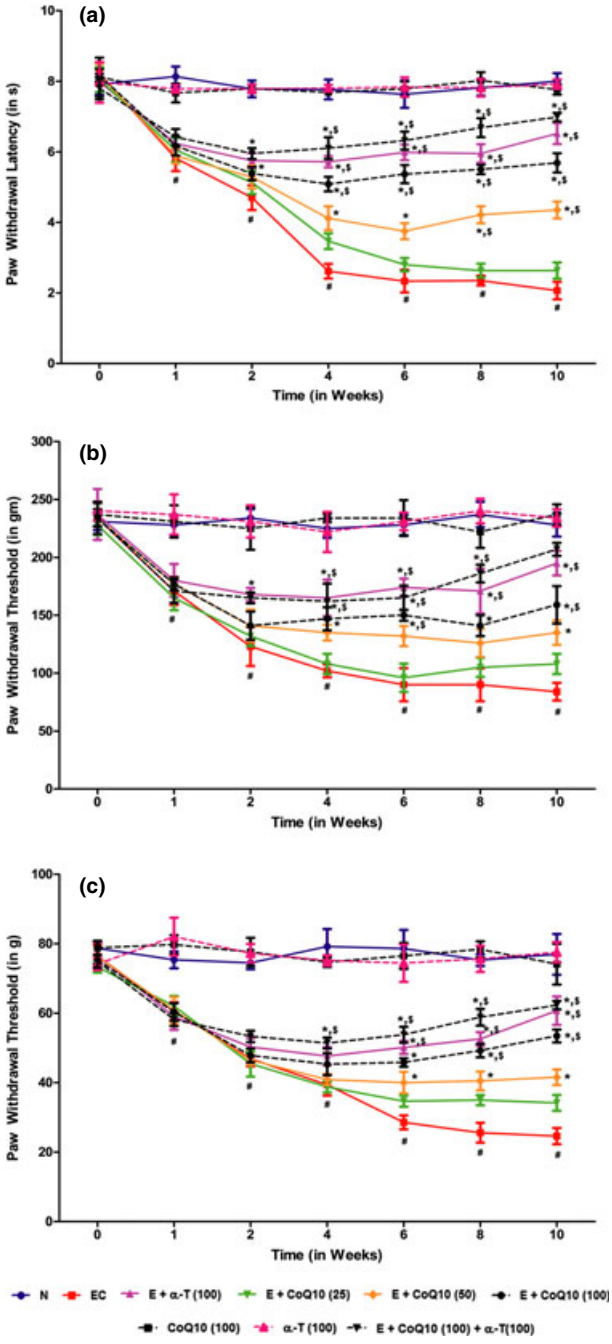

Figure 2 Effect of Coenzyme Q10 and α-tocopherol treatment on thermal hyperalgesia (a), mechanical hyperalgesia (b) and mechano-tactile allodynia (c) in ethanol treated rats. Data are expressed as mean ± SEM (n = 6) and analyzed by two way ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ as compared to the ethanol control group, $^{\#}P < 0.05$ as compared to normal group and $^{\$}P < 0.05$ as compared to one another. N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg) treated rats; E + CoQ10 (25), ethanol + Coenzyme Q10 (25 mg/kg) treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg) treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg) treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination treated rats; α-T (100), α-tocopherol (100 mg/kg) alone treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats

control rats (2.06 ± 0.24 s) as compared with normal rats (8.00 ± 0.22 s). Chronic treatment with Coenzyme Q10 (50 and 100 mg/kg) for 10 weeks showed significant and dose-dependent ($P < 0.05$) inhibition of decreased mean paw withdrawal latency (4.35 ± 0.24 and 5.68 ± 0.27 s, respectively) as compared with ethanol control rats. Rats received concomitant treatment with α-tocopherol (100 mg/kg) also showed significant attenuation ($P < 0.05$) of these decreased mean paw withdrawal latency (6.51 ± 0.29 s) when compared with ethanol control rats. However, rats treated with Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination showed more significant attenuation ($P < 0.05$) of the decreased mean paw withdrawal latency (6.98 ± 0.12 s) on 10th than Coenzyme Q10 or α-tocopherol alone treated groups. There was no significant change in mean paw withdrawal latency in normal as well as per se group, that is, Coenzyme Q10 (100 mg/kg, 7.76 ± 0.14 s) and α-tocopherol (100 mg/kg, 7.91 ± 0.13 s) over the same time period (*Figure 2a*).

Effect of Coenzyme Q10 on mechanical hyperalgesia

The mean paw withdrawal threshold on 0th day in ethanol control rats (234.00 ± 13.92 g) did not significantly differ as compared with normal rats (231.00 ± 7.64 g). Chronic ethanol administration for 10 weeks significantly decreased ($P < 0.05$) mean paw withdrawal threshold in ethanol control rats (84.00 ± 7.64 g) as compared with normal rats (228.0 ± 9.95 g). Rats concomitant administered with Coenzyme Q10 (50 and 100 mg/kg) for 10 weeks significantly and dose dependently attenuated ($P < 0.05$) this decreased mean paw withdrawal threshold (135.00 ± 10.60 and 159.0 ± 16.15 g) as compared with ethanol control rats. Whereas treatment with α-tocopherol (100 mg/kg) also showed significant increase($P < 0.05$) in mean paw withdrawal threshold (195.00 ± 10.60 g) as compared with ethanol control rats. However, when compared with Coenzyme Q10 or α-tocopherol alone treated groups, concomitant administration of the Coenzyme Q10 (100 mg/kg) and

α-tocopherol (100 mg/kg) combination showed more significant attenuation ($P < 0.05$) in decreased mean paw withdrawal threshold (207.00 ± 5.61 g). Normal as well as per se group, that is, Coenzyme Q10 (100 mg/kg, 237.00 ± 8.74 g) and α-tocopherol (100 mg/kg, 234.00 ± 7.64 g) did not showed any significant change in mean paw withdrawal threshold over the same time period (*Figure 2b*).

Effect of Coenzyme Q10 on mechano-tactile allodynia

There was no significant difference in the mean paw withdrawal threshold in ethanol control rats (76.44 ± 3.44 g) as compared with normal rats (78.69 ± 1.91 g) before administration of the ethanol. Chronic ethanol administration for 10 weeks significantly reduced ($P < 0.05$) mean paw withdrawal threshold (24.62 ± 2.32 g) as compared with the normal animals (76.94 ± 5.87 g). Concomitant administration of the Coenzyme Q10 (50 and 100 mg/kg) for 10 weeks significantly and dose dependently increased ($P < 0.05$) the mean paw withdrawal threshold (41.56 ± 2.22 and 53.46 ± 1.84 g) as compared with ethanol control rats. Whereas treatment with α-tocopherol (100 mg/kg) also significantly inhibited ($P < 0.05$) this reduced mean paw withdrawal threshold (60.74 ± 4.10 g) when compared with ethanol control rats. Concomitant administration of the Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination showed more significant increased ($P < 0.05$) in mean paw withdrawal threshold (62.30 ± 1.19 g) as compared with Coenzyme Q10 or α-tocopherol alone treated groups. The mean paw withdrawal threshold did not showed any significant change in normal as well as per se group over the same time period (*Figure 2c*).

Effect of Coenzyme Q10 on thermal hyperalgesia (tail immersion test)

On day 0, the mean tail withdrawal latency in ethanol control rats (14.22 ± 0.39 s) did not significantly differ than in normal rats (14.76 ± 0.22 s). Chronic administration of the ethanol for 10 weeks resulted significant decrease ($P < 0.05$) in the tail withdrawal latency (3.78 ± 0.41 s) as compared with normal rats (14.20 ± 0.56 s). Tail withdrawal latency in the Coenzyme Q10 (50 and 100 mg/kg) concomitant administered rats was significantly increased (7.52 ± 0.55 and 10.34 ± 0.27 s, respectively, $P < 0.05$) as compared with ethanol control rats. Also, there was significant increased ($P < 0.05$) in the tail withdrawal latency (10.92 ± 0.47 s) in α-tocopherol (100 mg/kg)-treated rats when compared with ethanol control rats. Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination-treated rats showed significant attenuation ($P < 0.05$) of these decreased tail withdrawal latency (11.36 ± 0.96 s) as compared with Coenzyme Q10 or α-tocopherol alone treated groups. There was no significant change in tail withdrawal latency in normal as well as per se group over the same time period (*Figure 3a*).

Effect of Coenzyme Q10 on motor co-ordination test

There was no significant difference in the mean fall of time (44.53 ± 1.19 s) in the ethanol control rats when compared with normal rats (43.55 ± 1.39 s). The mean fall of time in the ethanol control rats was significantly decreased (13.72 ± 1.10 s, $P < 0.05$) after 10 weeks of ethanol administration as compared with normal rats (44.92 ± 0.79 s). Concomitant treatment with Coenzyme Q10 (50 and 100 mg/kg) significantly and dose dependently ($P < 0.05$) increased the mean fall of time (25.56 ± 1.23 and 34.12 ± 1.40 s, respectively) as compared with ethanol control rats. When compared with ethanol control rats, treatment with α-tocopherol (100 mg/kg) also significantly attenuated ($P < 0.05$) this decreased mean fall of time (35.44 ± 1.02 s). Moreover, mean fall of time was more significantly attenuated (37.90 ± 1.12 s, $P < 0.05$) by the concomitant administration of Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination as compared with Coenzyme Q10 or α-tocopherol alone treated groups. The mean fall of time did not showed any significant change in normal as well as per se group over the same time period (*Figure 3b*).

Effect of Coenzyme Q10 on MNCV

Motor nerve conduction velocity in the ethanol control rats (55.26 ± 1.94 m/s) on day 0 did not significantly differ as compared with normal rats (56.59 ± 1.72 m/s). Chronic ethanol administration for 10 weeks resulted significant reduction ($P < 0.05$) in the MNCV (21.54 ± 1.42 m/s) in ethanol control rats as compared with normal rats (56.62 ± 1.65 m/s). Chronic treatment with Coenzyme Q10 (50 and 100 mg/kg) significantly and dose dependently increased ($P < 0.05$) the MNCV (34.10 ± 2.72 and 45.06 ± 1.40 m/s, respectively) as compared with ethanol control rats. There was significant increase ($P < 0.05$) in the MNCV (45.94 ± 1.83 m/s) in α-tocopherol (100 mg/kg) treated rats

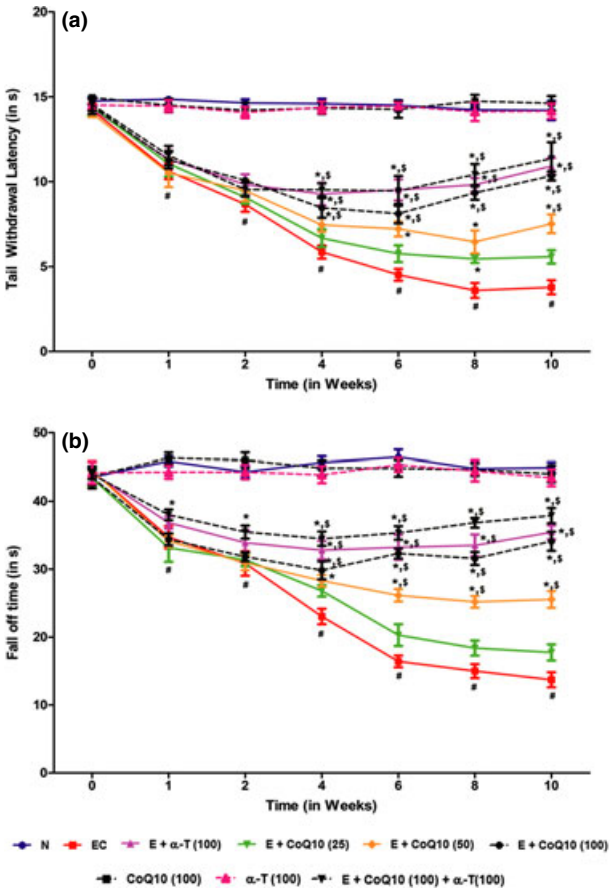

Figure 3 Effect of Coenzyme Q10 and α-tocopherol treatment on thermal hyperalgesia (tail immersion test) (a), and motor co-ordination test (b) in ethanol treated rats. Data are expressed as mean ± SEM (n = 6) and analyzed by two way ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ as compared to the ethanol control group, $^{\#}P < 0.05$ as compared to normal group and $^{\$}P < 0.05$ as compared to one another. N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg) treated rats; E + CoQ10 (25), ethanol + Coenzyme Q10 (25 mg/kg) treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg) treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg) treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination treated rats; α-T (100), α-tocopherol (100 mg/kg) alone treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats.

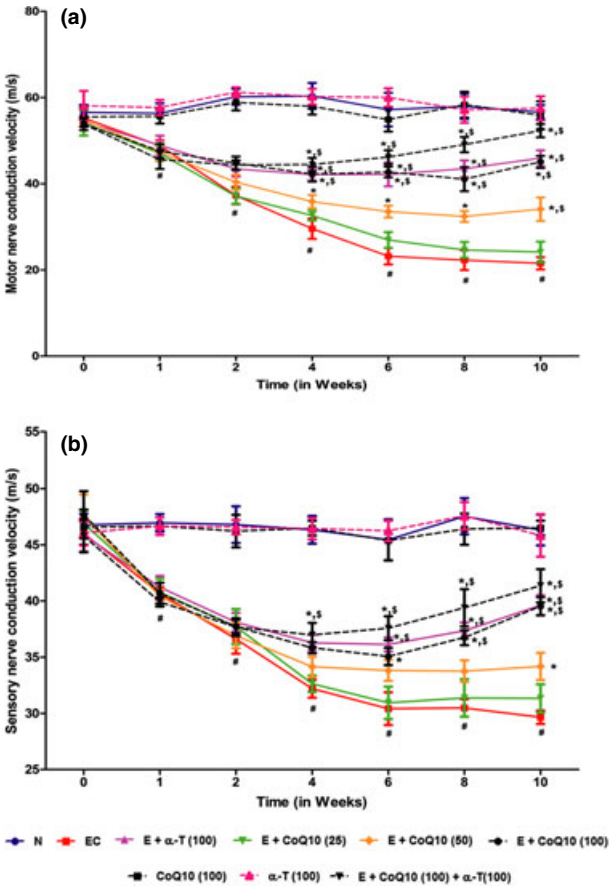

Figure 4 Effect of Coenzyme Q10 and α-tocopherol treatment on motor nerve conduction velocity (a), and sensory nerve conduction velocity (b) in ethanol treated rats Data are expressed as mean ± SEM (n = 6) and analyzed by two way ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ as compared to the ethanol control group, $^{\#}P < 0.05$ as compared to normal group and $^{\$}P < 0.05$ as compared to one another. N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg) treated rats; E + CoQ10 (25), ethanol + Coenzyme Q10 (25 mg/kg) treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg) treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg) treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination treated rats; α-T (100), α-tocopherol (100 mg/kg) alone treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats.

when compared with ethanol control rats. Moreover, this reduction in the MNCV was more significantly attenuated (52.30 ± 1.54 m/s, $P < 0.05$) by the concomitant administration of Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination as compared with Coenzyme Q10 or α-tocopherol alone treated groups. Normal as well as per se group, that is, Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) did not showed any significant change in MNCV over the same time period (*Figure 4a*).

Effect of Coenzyme Q10 on SNCV

There was no significant difference in SNCV of ethanol control rats (46.00 ± 1.04 m/s) on 0th week as

Table II Effect of Coenzyme Q10 on ethanol-induced alterations in SOD, GSH, MDA, NO, Calcium, and Na^+,K^+ATPase levels.

Treatment	SOD (U/mg of protein)	GSH (μg/mg protein)	MDA (nM/mg of protein)	NO (μg/mL)	Ca (ppm/mg of protein)	Na^+,K^+ATP ase (μmol/mg of protein)
Normal (N)	30.67 ± 0.88	1.34 ± 0.10	2.04 ± 0.32	109.4 ± 10.67	5.82 ± 0.38	12.24 ± 0.67
Ethanol control (EC)	3.89 ± 0.52$^{\#}$	0.20 ± 0.02$^{\#}$	10.17 ± 0.34$^{\#}$	341.4 ± 7.51$^{\#}$	23.38 ± 0.72$^{\#}$	3.67 ± 0.53$^{\#}$
E + α-T (100)	22.72 ± 0.71*,$	1.04 ± 0.02*,$	4.14 ± 0.35*,$	164.6 ± 7.86*,$	10.64 ± 0.42*,$	8.93 ± 0.47*,$
E + CoQ10 (25)	6.52 ± 0.50	0.40 ± 0.02	9.03 ± 0.47	326.1 ± 11.47	21.50 ± 0.49	4.37 ± 0.55
E + CoQ10 (50)	13.78 ± 1.22*,$	0.67 ± 0.03*,$	7.16 ± 0.54*	273.9 ± 6.82*	17.23 ± 0.60*,$	6.74 ± 0.65*
E + CoQ10 (100)	21.04 ± 1.12*,$	0.93 ± 0.03*,$	5.63 ± 0.31*,$	202.3 ± 10.73*,$	13.42 ± 8.73*,$	8.29 ± 0.46*,$
E + CoQ10 (100) + α-T (100)	26.64 ± 1.25*,$	1.13 ± 0.04*,$	3.71 ± 0.42*,$	137.8 ± 7.39*,$	8.73 ± 0.72*,$	9.97 ± 0.53*,$
CoQ10 (100)	30.68 ± 0.84	1.32 ± 0.04	2.82 ± 0.28	113.7 ± 10.53	6.69 ± 0.40	11.38 ± 0.36
α-T (100)	29.52 ± 1.00	1.30 ± 0.01	3.08 ± 0.19	112.5 ± 10.50	6.64 ± 0.59	11.34 ± 0.62

N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg)-treated rats; E + CoQ10 (25), ethanol + Coenzyme Q10 (25 mg/kg)-treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg)-treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg)-treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination-treated rats; α-T (100), α-tocopherol (100 mg/kg) alone treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; NO, nitric oxide.

Data are expressed as mean ± SEM (n = 6) and one way ANOVA followed by Tukey's multiple range test.

*P < 0.05 as compared with the ethanol control group, $^{\#}P$ < 0.05 as compared with normal group and $^{\$}P$ < 0.05 as compared with one another.

compared with normal rats (46.76 ± 1.02 m/s). Chronic ethanol administration resulted significant reduction (P < 0.05) in the SNCV in ethanol control rats (29.66 ± 0.59 m/s) as compared with normal rats (46.32 ± 1.37 m/s). Rats treated with Coenzyme Q10 (50 and 100 mg/kg) showed significant and dose-dependent inhibition (P < 0.05) of this decreased SNCV (34.18 ± 1.20 and 39.54 ± 0.79 m/s, respectively) as compared with ethanol control rats. However, treatment with α-tocopherol (100 mg/kg) also exhibits similar significant increased (P < 0.05) in the SNCV (39.62 ± 0.89 m/s) as compared with ethanol control rats. On the other hand, rats treated with Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination showed more significant attenuation (P < 0.05) of the decreased SNCV (41.36 ± 1.47 m/s) as compared with Coenzyme Q10 or α-tocopherol alone treated groups. The SNCV did not showed any significant change in normal as well as per se group over the same time period (*Figure 4b*).

Effect of Coenzyme Q10 on SOD level

Chronic ethanol administration for 10 weeks resulted significant (P < 0.05) decrease in the level of SOD in ethanol control rats as compared with normal rats. Treatment with Coenzyme Q10 (50 and 100 mg/kg) showed significant and dose dependently increased (P < 0.05) in the level of SOD as compared with ethanol control rats. However, treatment with α-tocopherol (100 mg/kg) also showed significant attenuation (P < 0.05) of the decreased level of SOD as compared with ethanol control rats. Moreover, this decreased level of SOD was more significantly attenuated (P < 0.05) by the treatment of combination of Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) than Coenzyme Q10 or α-tocopherol alone treated groups. There was no significant change in the level of SOD in per se group over the same period of time (*Table II*).

Effect of Coenzyme Q10 on reduced GSH level

There was significant reduction (P < 0.05) in the level of reduced GSH after chronic administration of alcohol in ethanol control rats as compared with normal rats. Rats received treatment with Coenzyme Q10 (50 and 100 mg/kg) showed significant inhibition (P < 0.05) of these decreased level of GSH as compared with ethanol control rats. However, combined treatment with Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) showed more increased (P < 0.05) in the level of GSH as compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone. The GSH level in per se group did not showed any significant change over the same period of time (*Table II*).

Effect of Coenzyme Q10 on lipid peroxidation level

The level of lipid peroxidation (MDA) in the ethanol control rats was significantly elevated (P < 0.05) after the chronic administration of ethanol as compared

with normal rats. Concomitant treatment with Coenzyme Q10 (50 and 100 mg/kg) significantly and dose dependently ($P < 0.05$) decreased the level of MDA as compared with ethanol control rats. The reduced level of MDA was more significantly attenuated ($P < 0.05$) in the Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination-treated group as compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone (*Table II*).

Effect of Coenzyme Q10 on nitric oxide level

When compared with normal rats, the level of nitric oxide (NO) in the sciatic nerve of the ethanol control rats was significantly increased ($P < 0.05$) after 10 weeks of ethanol administration. Treatment with Coenzyme Q10 (50 and 100 mg/kg) showed significant reduction ($P < 0.05$) in the elevated level of NO as compared with ethanol control rats. Rats treated with α-tocopherol (100 mg/kg) also showed significant decreased ($P < 0.05$) in the level of NO when compared with ethanol control rats. The elevated level of NO was more significantly inhibited ($P < 0.05$) by the concomitant combine treatment of Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) as compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone treated group. There was no significant change in the level of NO in per se group over the same period of time (*Table II*).

Effect of Coenzyme Q10 on total calcium content

Chronic administration of ethanol for 10 weeks resulted significant increased ($P < 0.05$) in the level of calcium in ethanol control rats as compared with normal rats. Treatment with Coenzyme Q10 (50 and 100 mg/kg) significantly attenuated ($P < 0.05$) this elevated levels of calcium as compared with ethanol control rats. This inhibition of elevated level of calcium was more significant ($P < 0.05$) in Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination-treated group as compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone treated group. The calcium level in per se group did not showed any significant reduction over the same period of time (*Table II*).

Effect of Coenzyme Q10 on membrane-bound inorganic phosphate (Na^+,K^+ATPase) level

The level of membrane-bound inorganic phosphate (Na^+, K^+ATPase) in ethanol control rats was significantly decreased ($P < 0.05$) as compared with normal rats. This decreased level of Na^+,K^+ATPase was significantly and dose dependently ($P < 0.05$) inhibited by the treatment of Coenzyme Q10 (50 and 100 mg/kg) as compared with ethanol control rats. However, treatment with α-tocopherol (100 mg/kg) also showed significant attenuation ($P < 0.05$) in the decreased level of Na^+,K^+ATPase as compared with ethanol control rats. Moreover, rats treated with combination of Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) showed more significant increased ($P < 0.05$) in the level of Na^+,K^+ATPase as compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone treated group. There was no significant change in the level of membrane-bound inorganic phosphate in per se group over the same period of time (*Table II*).

Effect of Coenzyme Q10 on TNF-α and IL-1β level

The level of pro-inflammatory cytokine, that is, TNF-α and IL-1β were significantly elevated ($P < 0.05$) in the rats treated with ethanol after 10 weeks as compared with normal rats. Treatment with Coenzyme Q10 (50 and 100 mg/kg) showed significant and dose dependently ($P < 0.05$) decreased in the elevated levels of pro-inflammatory cytokine as compared with ethanol control rats. Rats treated with α-tocopherol (100 mg/kg) also showed significant inhibition ($P < 0.05$) of the elevated levels of TNF-α and IL-1β as compared with ethanol control rats. When compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone treated group, Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination-treated rats showed more significant decreased ($P < 0.05$) in the level of pro-inflammatory cytokine. The level of pro-inflammatory cytokine, that is, TNF-α and IL-1β were remained unchanged in the per se group over the same period of time (*Table III*).

Effect of Coenzyme Q10 on IL-4 level

Chronic administration of ethanol for weeks resulted significant increased ($P < 0.05$) in the level of IL-4 in ethanol control rats as compared with normal rats. When compared with the ethanol control rats, Coenzyme Q10 (50 and 100 mg/kg) showed significant and dose-dependent ($P < 0.05$) attenuation in the elevated levels of IL-4. Whereas, rats treated with α-tocopherol (100 mg/kg) also showed significant decrease ($P < 0.05$) in the level of IL-4 as compared with ethanol control rats. Rats treated with Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) combination showed more significant attenuation ($P < 0.05$) in the elevated levels of IL-4 as compared with Coenzyme

Table III Effect of Coenzyme Q10 on ethanol-induced alterations in TNF-α, IL-1β, and IL-4 levels.

Treatment	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-4 (pg/mL)
Normal (N)	56.52 ± 3.72	20.16 ± 1.50	23.80 ± 3.33
Ethanol control (EC)	169.7 ± 5.23$^{\#}$	72.06 ± 2.51$^{\#}$	109.2 ± 7.25$^{\#}$
E + α-T (100)	83.56 ± 4.15$^{*,\$}$	28.44 ± 2.16$^{*,\$}$	43.00 ± 3.52$^{*,\$}$
E + CoQ10 (25)	155.7 ± 5.78	63.68 ± 3.33	99.12 ± 4.02
E + CoQ10 (50)	128.1 ± 5.92$^{*,\$}$	49.30 ± 4.27$^{*,\$}$	84.22 ± 3.25*
E + CoQ10 (100)	102.2 ± 4.13$^{*,\$}$	38.04 ± 2.73$^{*,\$}$	61.78 ± 2.74$^{*,\$}$
E + CoQ10 (100) + α-T (100)	69.7 ± 3.08$^{*,\$}$	23.78 ± 1.90$^{*,\$}$	38.68 ± 3.24$^{*,\$}$
CoQ10 (100)	59.4 ± 6.43	20.90 ± 1.28	26.14 ± 3.19
α-T (100)	59.30 ± 8.87	22.58 ± 1.44	24.82 ± 3.12

N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg)-treated rats; E + CoQ10 (25), ethanol + Coenzyme Q10 (25 mg/kg)-treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg)-treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg)-treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination-treated rats; α-T (100), α-tocopherol (100 mg/kg) alone treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats.

Data are expressed as mean ± SEM (n = 6) and one way ANOVA followed by Tukey's multiple range test.

*P < 0.05 as compared with the ethanol control group, $^{\#}P$ < 0.05 as compared with normal group and $^{\$}P$ < 0.05 as compared with one another.

Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone treated group. There was no significant change in the level of IL-4 in per se group over the same period of time (*Table III*).

Effect of Coenzyme Q10 on protein expression level of pol γ

The pol γ is a gene responsible for protective enzyme. The protein level of pol γ was significantly reduced ($P < 0.05$) in the ethanol control rats as compared with normal rats. The decreased level of pol γ represented minimum activity of the repair enzymes. Rats treated with Coenzyme Q10 (50 and 100 mg/kg) showed significant and dose-dependent attenuation ($P < 0.05$) of the decreased level pol γ as compared with ethanol control rats. Treatment with α-tocopherol (100 mg/kg) also showed significant increased ($P < 0.05$) in the expression of the pol γ as compared with ethanol control rats. However, this expression of the pol γ was more significantly increased ($P < 0.05$) in the rats treated with the combination of Coenzyme Q10 (100 mg/kg) and α-tocopherol (100 mg/kg) compared with Coenzyme Q10 (100 mg/kg) or α-tocopherol (100 mg/kg) alone treated group (*Figure* 5).

DISCUSSION

Peripheral polyneuropathy bearing close resemblance to human neuropathic pain is induced in laboratory animals by chronic administration of ethanol over a period of 10 weeks [6,7,47]. The prevalence of alcohol-induced neuropathic pain due to chronic alcohol consumption is about 9–50% worldwide [48]. Malnutrition, vitamins B_1 deficiency, toxicity of alcohol, and family history of alcoholism are some of the several risk factors that are associated with alcohol-induced neuropathic pain [3,48].

Ethanol is degraded by ethanol dehydrogenase and acetaldehyde dehydrogenase into acetaldehyde, which attached with axon and cyton of the peripheral neuron initiating a vicious cycle disturbing the energy metabolism

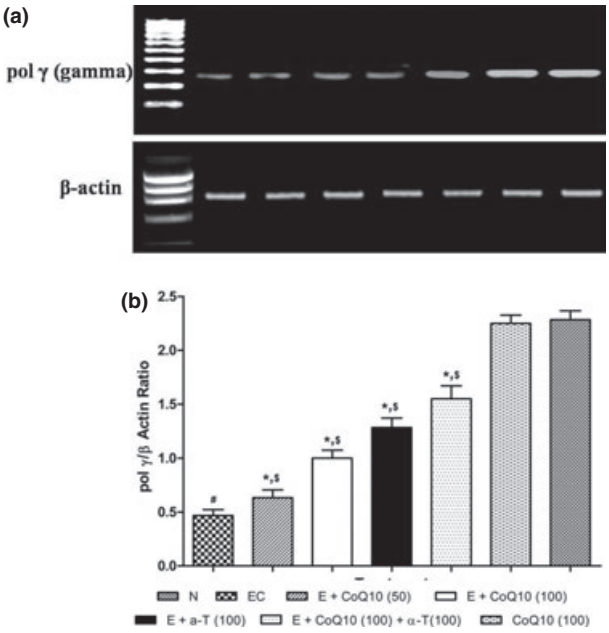

Figure 5 Effect of Coenzyme Q10 on reverse transcriptase analysis of protein expression of polymerase gamma (pol γ) (a) Quantitative representation of protein expression of pol γ (b) Data are expressed as mean ± SEM (n = 6) and one way ANOVA followed by Tukey's multiple range test. *P < 0.05 as compared to the ethanol control group, $^{\#}P$ < 0.05 as compared to normal group and $^{\$}P$ < 0.05 as compared to one another. N, normal rats; EC, ethanol control rats; E + α-T (100), ethanol + α-tocopherol (100 mg/kg) treated rats; E + CoQ10 (50), ethanol + Coenzyme Q10 (50 mg/kg) treated rats; E + CoQ10 (100), ethanol + Coenzyme Q10 (100 mg/kg) treated rats; E + CoQ10 (100) + α-T (100), ethanol + Coenzyme Q10 (50 mg/kg) + α-tocopherol (100 mg/kg) combination treated rats; CoQ10 (100), Coenzyme Q10 (100 mg/kg) alone treated rats.

oxidative balance and electron transport chain. It causes elevation in the level of oxidative biomarkers, including thiobarbituric acid-reactive substances (TBRAS), hydrogen peroxide, and OH^- like species [49,50]. This biochemical alteration leads to chronic peripheral pain [51]. Axonal damage and demylination of motor as well as sensitive fibers are irreversible and can be behaviorally measured by elevated allodynia, hyperalgesia, and motor in-coordination of the limbs in laboratory animals [52].

Chronic alcohol consumption diminished the pain threshold of nociceptor located on the peripheral sensory and motor neurons [53,54]. This is quantified by Randall sellitto and von Frey hair, which measure mechanical hyperalgesia and tactile allodynia. Rats with long-term ethanol consumption demonstrate enhanced nociception due to release of inflammatory mediators like bradykinin, NGF, ATP, PGE2 with TRPV1 ion channel [55]. Coenzyme Q10 restores the reduced threshold due to chronic alcohol consumption, thus alleviating of neuropathic pain.

In conjugation with allodynia and hyperalgesia, chronic alcohol consumption also leads to disregulation of thermal receptor causing a decline in the threshold of response to thermal stimuli [56,57]. This was evident in the Hargreaves test/plantar test. Finding of present investigation is in consistent with the previous reports Dina et al. and Miyoshi et al. [4,52]. Coenzyme Q10 demonstrates marked increased in reduced pain threshold to heat stimulus in the peripheral neurons.

Motor in-coordination is a behavioral expression of neuropathic dysfunction due to alteration in the soleus and extensor digitorum longus (EDL) muscles tone [58]. It can be measured by elevating grip strength of neuropathic animals. Peripheral motor in-coordination was restored by treatment of Coenzyme Q10, as it halted the development of neuropathy by preventing structural destruction of neuron makeup.

Activation of nociception pathways, which originate in tail, has been investigated to be dependent on the stimulus temperature [59]. This can be measured by either radiant heat test or tail immersion test. Noxious thermal stimuli elicits pain response when immerse in 55 °C water. It is a spinal motor reflex, and it closely associated with neuronal dysfunction [60]. Coenzyme Q10 reverses the sensory damage and hyperalgesia response in tail immersion test produce by the ethanol.

Chronic alcohol consumption leads to electrophysiological modification due to ethanol intoxication. The pathological changes including fiber degeneration, demyelination, axonal shrinkage, and endoneural edema lead to conduction dysfunction of nerve [61,62]. The motor and sensory neurons are simultaneously affected, and velocity of nerve conduction is decreased due to damage to neuronal ultra structure [63]. The biochemical and inflammatory mediators are responsible for decreased of MNCV as well as SNCV [64,65]. Coenzyme Q10 significantly abrogated alcohol-induced downregulation of conduction velocity. This property of Coenzyme Q10 may be attributed to its potent antioxidant profile.

Electron transport chain is an indispensible physiological component maintaining the mitochondrial energy metabolism, and long-term ethanol consumption has been associated with increased in ROS with concomitant disregulation of cellular redox balance. The biological properties of axonal enzyme and protein are altered by acetaldehyde, which is a major neurotoxic metabolite progressively delapilating protein, lipid, and DNA nerve cell [10,66–68]. SOD is an essential enzyme, which is responsible for maintanance of redox balance in the neuron as well as vascular endothelial damage [69]. Increased in the Superoxide anions resulted in activation of aldose reductase and protein kinase C, which are further resulted in pain perception [14]. Under oxidative stress, the level of SOD is diminished reflecting nerve damage. The elevated level of superoxide anions was diminished by SOD, as it transforms these anions to H_2O_2. Coenzyme Q10 was able to significantly elevate the depreciated levels of SOD.

Glutathione is an important quencher of electron, and its level is reduced under oxidative stress [70,71]. GSH S-transferase is an enzyme that responsible for detoxification of electrophiles. It has been well documented that decreased level of GSH leads to cellular damage [7,72], which intern causes hyperalgesia [70]. Coenzyme Q10 was able to increase the level of GSH reiterating its antioxidant profile.

Endoneural oxidative stress is depicted by elevated levels of MDA. Neuronal antioxidant enzymes are unable to reduce the oxidative injury leading to generation of free radicals in cytoplasm [73]. Elevated levels of MDA correspond to increased oxidative stress in the neurons due to structural destruction of unsaturated fatty acids in lipid membrane [74]. Chronic ethanol administration resulted generation and maintenance of oxidative stress, which plays a pivotal role in the development of alcoholic neuropathy. Our study agrees with the findings of the Tiwari et al. and Raygude et al. [7,65]. Coenzyme Q10 inhibited the uncontrolled rise of

MDA level by dents of its antioxidant profile. Result of present investigation is in tuned with the findings of Bargossi et al. [75], which showed that administration of Coenzyme Q10 decreases concentration lipid peroxidation via downregulation of oxidative phosphorylation.

Coenzyme Q10 is an essential cofactor of the electron transport chain as well as an endogenous antioxidant, which is reduced due to oxidative insult, and this leads to mitochondrial respiratory dysfunction [76]. It has been previously reported that activity of the electron transport chain was increased by concomitant administration of Coenzyme Q10 [77]. Administration of Coenzyme Q10 exerts neuroprotective effects, as the depleted reserve of Coenzyme Q10 were replenished in the present investigation. It explains the neuroprotective profile of Coenzyme Q10 exhibited via behavioral as biochemical parameters. Result of present investigation is in accordance with the findings of Beal et al. [78], which showed in vivo neuroprotective effects of Coenzyme Q10 via attenuation of malonate-induced ATP depletions.

Exitotoxicity is an inevitable phenomenon accompanying unfettered oxidative stress [6,65]. Elevated levels of calcium demonstrated enhanced oxidative stress. This leads to a cascade of biochemical changes, causing degradation and degeneration of axonal cytoskeleton [79]. There is a simultaneous release of free radical precipitating neuronal dysfunction. The elevated level of calcium in chronic ethanol administration demonstrates its vital role in the alcohol-induced neuropathic pain. Coenzyme Q10 prohibited the elevation of calcium in sciatic nerve, thus preventing the neuronal damage.

Production of nitric oxide is invariably coupled with deficient level of SOD resulting in production of peroxynitrite, which intern sensitizes spinal neurons. Abnormalities of NO production in endoneural and epineural microvasculature leads to reduced perfusion and hypoxia [63,80–82]. Nitric oxide is also implicated in neuropathic pain by sensitizing spinal neurons and central neurons [83]. These changes were hindered by administration of Coenzyme Q10 as it inhibited surge of NO, and thus, exhibiting neuroprotective action. The results of the present investigation provide credence to the study carried out by previous author Oztay et al. and Jung et al. [84,85].

Membrane-bound inorganic phosphate enzymes, that is, Na,K-ATPase play a pivotal role in maintaining electrolyte balance of nerve [34,86]. The conduction velocity of nerve varies directly with the level of Na, K-ATPase. ROS have a derogatory effect on Na,K-ATPase located on the neurolema [87]. Reduced levels of Na,K-ATPase associated with activation of polyol pathways leading to inactivation of phosphate correspond to nerve damage [88]. Coenzyme Q10 was able to significantly restore the level of Na,K-ATPase provides the credence to its antioxidant profile.

Painful alcoholic neuropathy is associated with elevated levels of hyperalgesia in which protein kinase C (PKC) signaling plays pivotal role [4,89]. Inflammatory mediators such as TNF-α and IL-1β are the prime factors that are responsible for this elevated hyperalgesia. The levels of pro-inflammatory cytokines are elevated due to chronic administration of alcohol [6,7]. This is implicated to autotomy and dysregulation of neuronal activity. TNF-α and IL-1β contribute to the central sensitization and have been proven to initiate, maintain, and elevate in neuropathic pain [90]. It has been documented that release of TNF-α in sciatic nerve produces thermal hyperalgesia and mechanical allodynia [91,92]. Recently, Samad et al. [93] reported that inflammatory pain hypersensitivity arises due to overexpression of cyclo-oxygenase-2 induced by the release of IL-β. The level of TNF-α and IL-β were found to be reduced due to administration of Coenzyme Q10. Schmelzer et al. [22] showed that during inflammatory injury, treatment with Coenzyme Q10 significantly inhibit the release of pro-inflammatory cytokines. Result of the present investigation corroborate with the findings of Schmelzer et al. [22].

Reduced blood flow to neurons has been known to elevate the neurotoxicity and nociception [94]. Inflammatory cytokines especially IL-4 have been reported to be anti-angeogenic, thus increased hypoxia [95]. IL-4 levels were elevated in the group of animals devoid of Coenzyme Q10 treatment. This animals exhibited intern neurotoxic condition. Hence, our study is a pioneer investigation demonstrating the underline relation between IL-4 and neurotoxicity. Te Velde et al. and Hart et al. [96,97] reported that release of IL-4 plays an important role in the TNF-α- and IL-1 β-induced inflammatory responses. It can be hypothesis that reduced angiogenesis and blood flow due to increase IL-4 level may have resulted in increased pain perception, which was inhibited by Coenzyme Q10 administration.

Mitochondrial DNA does not possess protection of histone and is proximal to electron transport chain, which is major source of (O_2) and hence is vernable to damage. Oxidative damage to mtDNA has been recently shown to be responsible for neurotoxicity [66].

Based excision repair (BER) pathway involved a programmed activation of DNA repair enzyme, which is responsible for neuroprotection and repairing oxidative neuronal DNA damage [98]. BER pathway is function in nucleus as well as mitochondria [99]. Neuronal survival is dependent upon equilibrium between oxidative damage and repair system. Upregulate ROS levels have been associated with reduced mtBER enzymes and neurotoxicity. The expression of pol γ corresponds to levels of BER enzymes [46]. The present investigation demonstrates reduced expression of BER in vehicle-treated animals, whereas elevated expression was evident in Coenzyme Q10-treated animals. DNA fragmentation is an index of degree of damage and cell death. There was market decreased in degree of DNA fragmentation in Coenzyme Q10-supplemented animals. Ethanol has been previously reported to cause apoptotic changes in the nerve [6,65]. Hence, present investigation demonstrates anti-apoptotic property of Coenzyme Q10. Quiles et al. [100] reported the anti-apoptotic potential of Coenzyme Q10 in vivo. Result of the present investigation is in tuned with the findings of Quiles et al. [100].

Vitamin E is a liquid-soluble potent antioxidant possessing anti-neuroinflammatory property [7]. It has been shown to prophylactically arrest neurotoxicological changes in various model of neuropathy [7,34]. Similar profile was exhibited by Coenzyme Q10 alone in the present investigation. This finding investigated us to concomitantly administer a combination of Coenzyme Q10 and vitamin E. Concomitant administration of combination of these two antioxidants was beneficial than Coenzyme Q10 or vitamin E alone treatment in alleviating the oxidative stress in alcohol-induced neuropathic pain. Previous study by Niki et al. [101] also showed that combination of CoenzymeQ10 with another antioxidant like vitamin E and vitamin C beneficial in increasing the antioxidant efficiency of the each other.

The pharmacotherapy of neuropathic pain has not advanced, and there are no approved therapies to improve the long-term prognosis of peripheral nerve injury [102]. Recent treatment regiment for neuropathic pain includes various natural antioxidant therapies. Along with rehabilitation therapy treatment of alpha-lipoic acid as well as gamma-linolenic acid for 6 weeks showed significant improvement in neuropathic symptoms in the patient with radicular neuropathy [103]. In another trail, 3 months of treatment of vitamin E (alpha-tocopherol 400 mg/day) showed significant protects against cisplatin-induced peripheral neuropathy [104]. Treatment with natural antioxidant like alpha-Lipoic Acid (600 mg/day), Acetyl-L-Carnitine (500 mg/day), and various vitamins including Thiamine, Methylcobalamin provided promising approach for treatment of peripheral neuropathic pain [105]. It has been documented that treatment with Coenzyme Q10 (60 mg twice daily) for 28 days in patients with coronary artery disease significantly reduced the elevated levels of thiobarbituric acid-reactive substances, malondealdehyde, and diene conjugates [106].

Thus, coadministration of Coenzyme Q10 and vitamin E resulted in prevention of behavioral, biochemical, and molecular neurotoxic effect of alcohol administration. This finding may open novel vistas in therapeutic option with natural antioxidants like vitamin E and Coenzyme Q10 to treat alcohol-induced neuropathic pain. It is evident from finding of present investigation that plethora of mechanism simultaneously orchestrate to exhibits neuroprotective effect of Coenzyme Q10, vitamin E, and their combination.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Elucidation of molecular mechanism involved in neuroprotective effect of Coenzyme Q10 in alcohol induced neuropathic pain.

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