Received: 28 August 2019 | Revised: 24 November 2019 | Accepted: 8 January 2020

DOI: 10.1002/jbt.22449

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Neuroprotective efficacy of nano-CoQ against propionic acid toxicity in rats: Role of BDNF and CREB protein expressions

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Funding information
Deanship of Scientific Research at King Saud University, Grant/Award Number: URSP – 4 – 19 – 19

Abstract: Propionic acid (PRA) is used as a food preservative. This study was aimed to investigate the neuroprotective effect of acetyl-L-carnitine (ALC) and nano-Coenzyme Q (N-CoQ) on brain intoxication induced by PRA in rats. Rats were divided into five groups: group I: control; group II: received PRA; group III: received ALC; group IV: received N-CoQ; and group V: received ALC and N-CoQ for 5 days. The antioxidants in question markedly ameliorated serum interleukin-1β and tumor necrosis factor-α, and brain NO, lipid peroxide, glutathione, and superoxide dismutase levels as well as protein expression of brain-derived neurotrophic factor (BDNF) and P-cyclic-AMP response element-binding protein (CREB) that were altered by a toxic dose of PRA, as well as histopathological alterations, including improvement of the cerebellum architecture. Interestingly, the combination therapy of ALC and N-CoQ achieved the most neuroprotective effect compared with monotherapies. The current study established that N-CoQ is considered as a useful tool to prevent brain injury induced by PRA. BDNF and CREB proteins are involved in both PRA neurotoxicity and treatment.

KEYWORDS
acetyl-L-carnitine, BDNF and P-CREB, nano-Coenzyme-Q, neuroprotective, propionic acid

1 | INTRODUCTION

Propionic acid (PRA) is a food preservative widely used as a fungicide and a bactericide.[1] The mechanism of its antimicrobial effect is due to its ability to decrease the pH, as an increase in acidity harmfully affects microorganisms. Although PRA has multiple applications, it can be toxic if used in an inappropriate way. It can cause toxicity in many organs, for example, the brain, liver, and stomach. It was documented that PRA causes a depletion of serotonin, inhibition of glutathione-s-transferase activity, and an increase of proinflammatory cytokine interferon-γ, as well as causing DNA damage.[2–4]

Acetyl-L-carnitine (ALC) plays an essential physiological role in transporting long-chain fatty acids over the internal mitochondrial membrane for their β-oxidation and ATP production in peripheral tissues. It translocates acetyl-Co-A into cytoplasm throughout the mitochondria. ALC is effectively transported through the blood-brain barrier and collected in neural cells.[5] A significant modulatory rule for ALC in neural function might be played through that ALC-intervened exchange of acetyl bunches for the synthesis of acetylcholine, and also by impacting signal transduction pathways and gene expression.[6] Moreover, ALC is a critical cofactor for peroxisomal oxidation, particularly for long-chain fatty acids.[7] It plays a significant role as a cofactor, in the transportation of free fatty acid from the cytosol to the mitochondria. Dietary supplementation of ALC, the biologically active form of L-carnitine, has an ameliorative effect for uremic patients and can enhance nerve conduction, neuropathic agony and increase immunity in diabetes patients. Moreover, it saves the life of patients suffering from essential ALC inadequacy.[8]

Coenzyme Q (CoQ) is an endogenous lipid-soluble compound; it has an important role as an antioxidant in mitochondria and lipid membranes.[9,10] CoQ10 acts as an essential cofactor in the electron

transport chain where it accepts electrons from complex I and II.[9,11] It is also an obligatory cofactor of mitochondrial uncoupling proteins, which regulate ATP production and reduce free radical generation.[12] In the current study, nano-Coenzyme Q (N-CoQ) was used, since nanoparticles can easily enter most cells and readily interact with the biomolecules, leading to enhancement of the bioavailability of the native compound.[13]

Because little is known about the molecular neurotoxicity of PRA, this initiates our interest to evaluate novel molecular pathways of protein expression of brain-derived neurotrophic factor (BDNF) and cyclic-AMP response element-binding protein (CREB) in PRA neurotoxicity and possible treatment. Moreover, the present study is conducted to evaluate the neuroprotective efficacy of the combination of N-CoQ and ALC in the rat's brain exposed to PRA toxicity. This is the first time N-CoQ has been used in managing PRA neurotoxicity in rats.

2 | MATERIALS AND METHODS

2.1 | Chemicals

PRA and ALC were purchased from Sigma-Aldrich Co. N-CoQ were obtained from LipoLife, Drakes Lane Industrial Estate, Drakes Lane, (UK).

2.2 | Experimental design

In this study, the use of animals was under strict compliance with the Scientific Research Ethics Committee, King Saud University, IRB No. KSU-SE-19-15.

Thirty adult male albino rats weighing 150 g were obtained from the Experimental Animal Center, King Saud University. The animals were allowed to adapt in the laboratory for one week under standard environmental conditions of temperature 22°C and natural light/dark cycle. They were given a standard rat pellet diet and distilled water ad libitum. Rats were divided into five groups, six rats each. Group I (control) rats received 1% carboxymethylcellulose. Group II rats were orally administered PRA 250 mg/kg/day.[14] Group III (PRA-intoxicated) rats were treated with ALC 100 mg/kg/day.[15] Group IV (PRA-intoxicated) rats were orally administered 10 mg/kg/day of N-CoQ.[16] Group V rats were orally administered a mixture of ALC and N-CoQ. All treatments with the agents in question will be given for 5 days along with PRA.

After the completion of the experiment, all rats were euthanized using CO_2 gas and the animals were killed by decapitation. Blood samples were collected for serum separation by centrifugation at 3000 rpm at 4°C for 15 minutes. Brains were collected and then homogenized in phosphate-buffered saline solution to yield 20% homogenates. The homogenates were centrifuged at 3000 rpm at 4°C for 30 minutes, and the supernatants were collected for further analysis. Three hemispheres from different animals were kept under nitrogen for Western blot analysis, the other parts were kept for histological examination in 10% formalin.

2.3 | Brain NO, malondialdehyde, glutathione, and superoxide dismutase

Brain superoxide dismutase (SOD) was evaluated using the kits purchased from Randox Laboratories. Brain malondialdehyde (MDA) and glutathione (GSH) levels were assessed following Mihara and Uchiyama[17] and Ellman[18] methods, respectively. NO was determined according to the method described by Moshage et al.[19]

2.4 | Determination of serum tumor necrosis factor-α and interleukin-1β levels

Serum tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels were estimated using ELISA kits obtained from research and development at Randox (R&D) Co.

2.5 | Western Blot

Western blot of BDNF and P-CREB proteins' expressions were performed according to the method of Tahrin et al.[20]

2.6 | Histological examination

Sections of the brain were cut and used for histopathological examination using hematoxylin and eosin (H&E) stain.

2.7 | Statistical analysis

All values were expressed as the mean ± standard error of the mean. The results were analyzed by one-way analysis of variance, followed by the Tukey test for multiple comparisons. Analyses were computed using SPSS for Windows (version 11); $P < .05$ was considered to represent a significant difference.

3 | RESULTS

PRA caused a significant elevation of the serum inflammatory biomarkers (IL-1β and TNF-α) compared to the control group ($P \leq .001$) (Figure 1). While treatment with ALC and N-CoQ significantly decreased their levels in comparison with the PRA group. The combination therapy of ALC and N-CoQ achieved the most downregulation in their levels compared to monotherapies of ALC or N-CoQ ($P \leq .001$) (Figure 1).

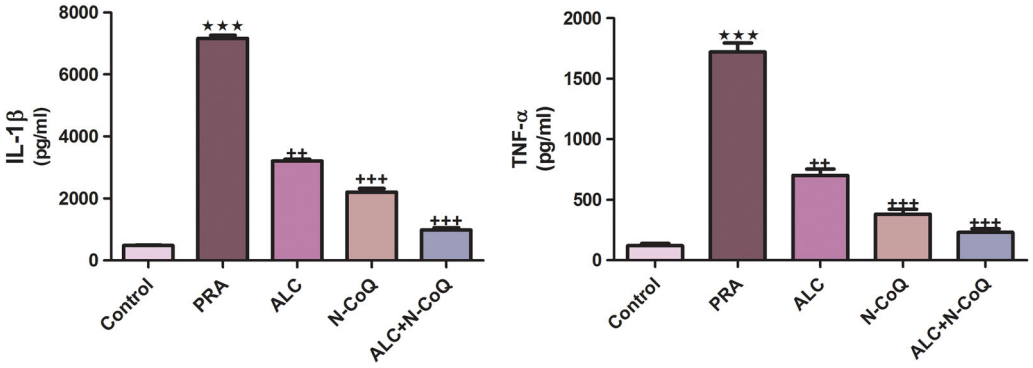

FIGURE 1 Serum levels of IL-1β and TNF-α in the control group as well as in all treated groups. Data are shown as mean ± SEM (N = 6). $^{***}P \leq .001$ vs control group, $^{++}P \leq .01$, $^{+++}P \leq .001$ vs administered-PRA group. IL, interleukin; PRA, propionic acid; SEM, standard error of the mean; TNF-α, tumor necrosis factor-α

Figure 2 shows that the levels of brain MDA and NO were significantly increased ($P \leq .001$), while GSH and SOD levels were decreased ($P \leq .001$) upon PRA intoxication. The administration of antioxidants in question improved all the previous measured parameters. Interestingly, the combination therapy of ALC and N-CoQ exhibited the most pronounced improvement in GSH, SOD, MDA, and NO levels compared with monotherapies with ALC or N-CoQ.

The expressions of BDNF and P-CREB proteins were significantly downregulated upon exposure to PRA comparing to the normal control group (Figure 3). Treatment with ALC and N-CoQ significantly ameliorated BDNF and P-CREB expressions in comparison with the PRA group (Figure 3).

Figure 4 presents light photomicrographs of H&E-stained sections of the brain. Brain sections of control rat revealed a normal architecture: a well-defined molecular, granular and Purkinje layers or pyramidal layer (Figure 4A). PRA intoxication caused alteration of the granular cell layer with a thin reduction in the cellular size of the molecular layer (Figure 4B). While the administration with ALC,N-CoQ or the combination therapy of ALC and N-CoQ caused improvement of the cerebellum architecture as shown in Figure 4C-E, the sections displayed almost normal

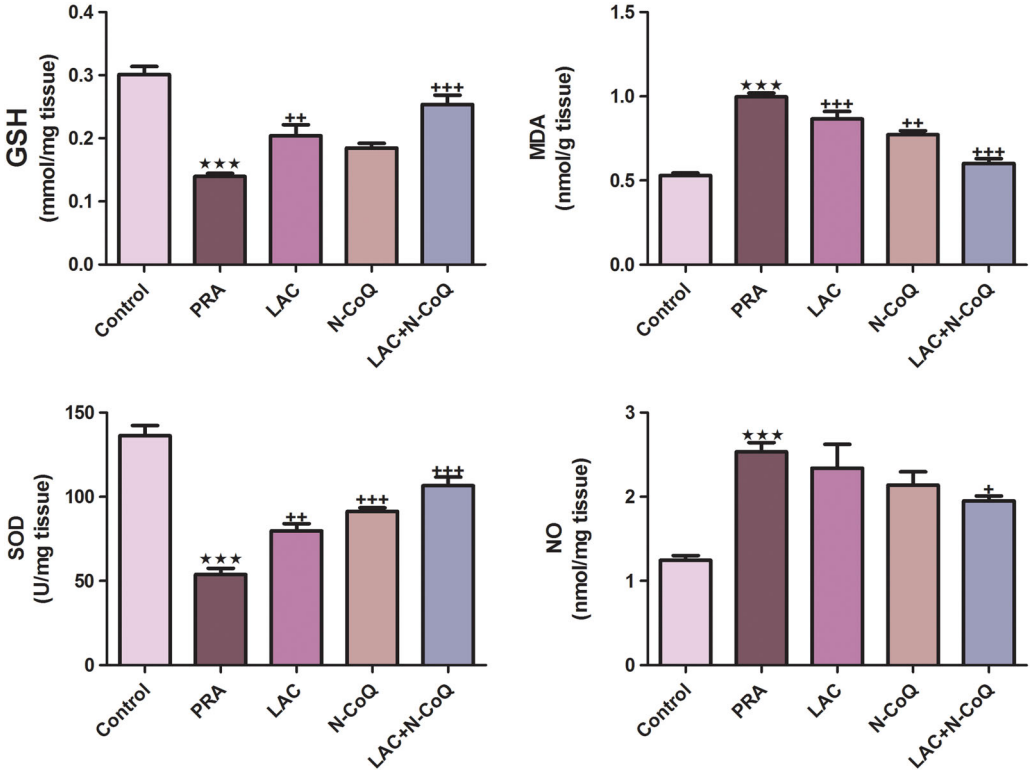

FIGURE 2 Oxidants/antioxidants biomarkers in the brain of the control group as well as in all treated groups. Data are shown as mean ± SEM (N = 6). $^{***}P \leq .01$ vs control group, $^{+}P \leq .05$, $^{++}P \leq .01$, $^{+++}P \leq .001$ vs administered-PRA group. N-CoQ, nano-Coenzyme Q; PRA, propionic acid; SEM, standard error of the mean

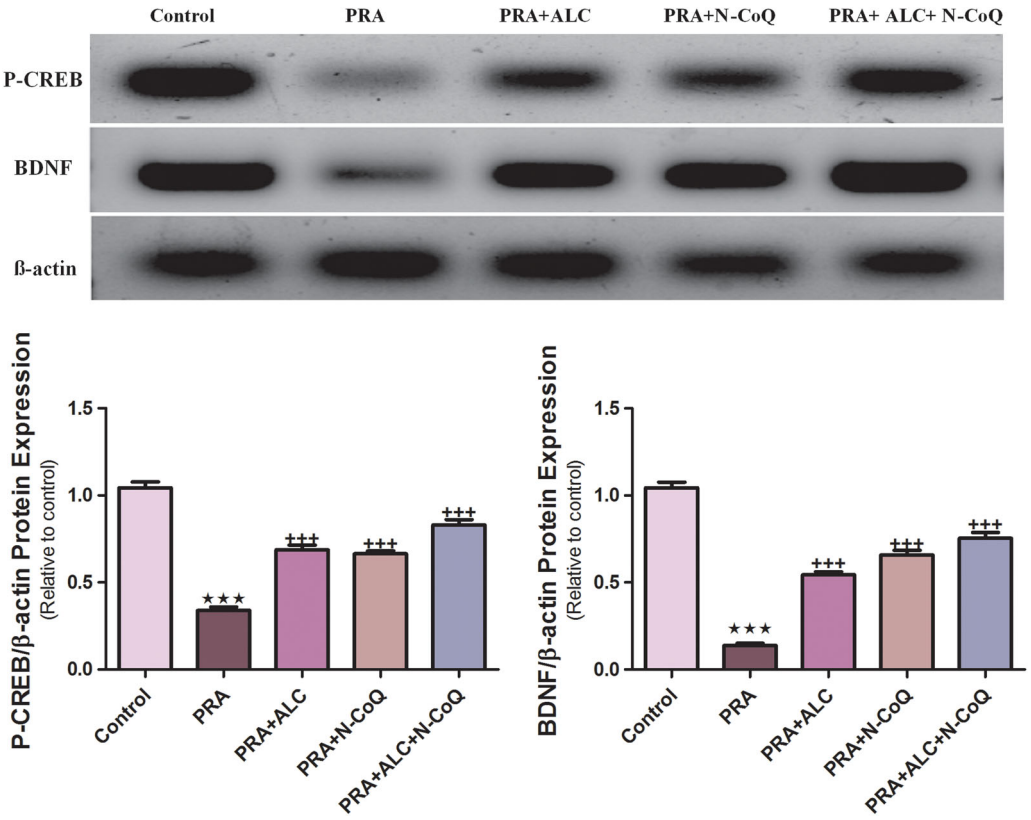

FIGURE 3 P-CREB and BDNF protein expression in the brain of the control group as well as in all treated groups. Data are shown as mean ± SEM (N = 6). $^{***}P \leq .01$ vs control group, $^{+++}P \leq .001$ vs administered-PRA group. BDNF, brain-derived neurotrophic factor; CREB, cyclic-AMP response element-binding protein; PRA, propionic acid; SEM, standard error of the mean

histological features, a well-defined molecular, granular and Purkinje layers or pyramidal layer.

4 | DISCUSSION

PRA is a food preservative; The antifungal mechanism of PRA is attributable to the inhibition of glucose metabolism by propionyl-CoA via the accumulation of the CoA derivatives.[21,22] The continuous administration of PRA may cause multiple harmful effects. High levels of PRA were reported to induce oxidative stress with decreased levels of total GSH in brain tissue.[23]

Previously, it was demonstrated that rat pups intoxicated by PRA exhibited elevation in the levels of IL-6 and TNF-α. Increased levels of such parameters could be easily correlated to brain injury.[24] These observations are parallel with our current study.

Herein, the administration of PRA caused downregulation in the expressions of BDNF and P-CREB proteins. BDNF has emerged as a key neurotrophin regulating synaptic plasticity, neuronal differentiation, and survival of new neurons and synapses.[25] BDNF depletion was reported in neurodegenerative and psychiatric disorders, associated with the severity of neurological dysfunction. In addition, the role of BDNF as a diagnostic marker in autism disorders has been reported before.[26] Moreover, CREB is a transcriptional factor that plays an important role in the synaptic connections' development.[27] These observations were compared with the results of the present work. Shared interactions between BDNF and P-CREB were well documented: BDNF promotes the phosphorylation of CREB, which, in turn, promotes the transcription of the BDNF gene.[28] BDNF activates CREB, in part, by increasing intracellular Ca^{2+}/calmodulin-dependent protein kinase type IV, which phosphorylates CREB.[29]

The results of the present study were similar to a study done by Mohmmad Abdul et al[30]; they revealed that ALC plays a protective role in cortical neuronal cells against amyloid-β peptide 1-42-mediated oxidative stress and neurotoxicity. In another study, ALC markedly increased the GSH and SOD in various organs.[31] Moreover, IL-1β, IL-6, and TNF-α were lowered in rat models of cachexia, septic shock by using ALC.[32] It counteracts oxidative stress, regulates NO release and improves the cellular respiration and activation of enzymes associated with resistance against oxidative stress.[33,34]

CoQ has an important role as an antioxidant in mitochondria and lipid membranes.[10,34] Defects in energy metabolism and oxidative damage play a role in the pathogenesis of neurodegenerative diseases[35,36]; hence, treatment with CoQ could exert many beneficial therapeutic effects.[37,38] In the current study, N-CoQ was used since nanoparticles can easily interact with biomolecules on both the surface and inside cells leading to

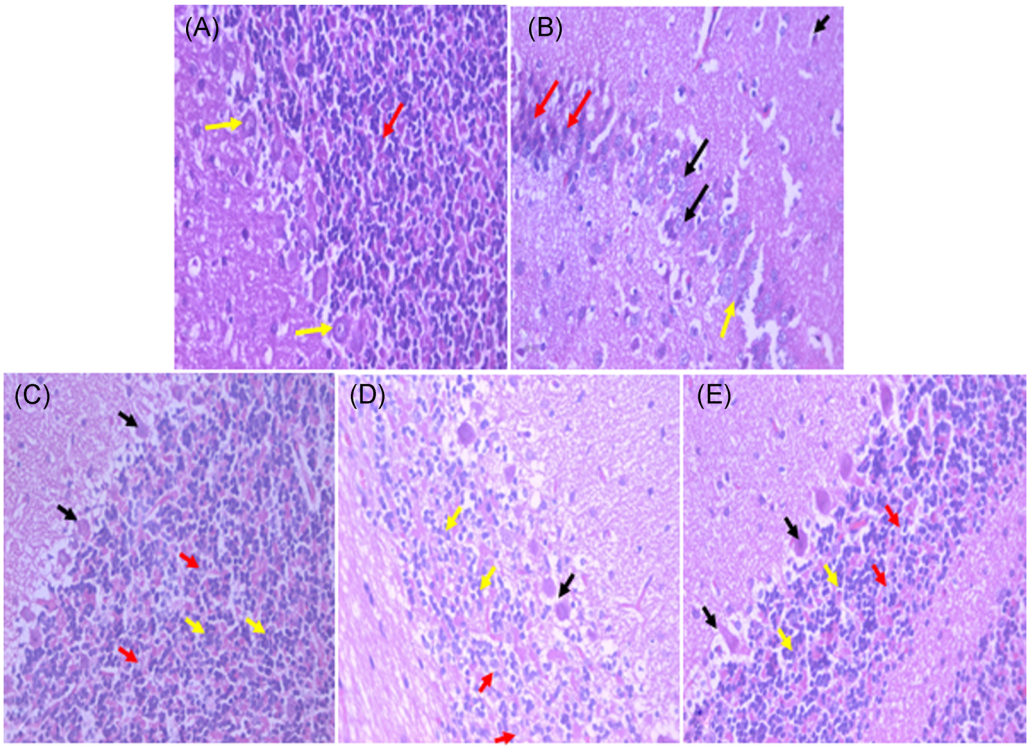

FIGURE 4 Light photomicrographs of brain sections stained with H&E stain, ×400. A, Brain section of normal control group, shows the cerebellum with normal histological features, granular (red arrow) and Purkinje layers or pyramid layer (yellow arrow). B, Brain section of a PRA group, shows the cerebellum with distortion of granular cell layer (red arrow), reduction in cellular size of the molecular layer, with few layers of large pyramid cells, also with vesicular nuclei (black arrow), also shows many glial cells among neuronal processes (black head). Scattered sparse cell distribution of Purkinje layers or pyramid cells with vesicular nuclei (yellow arrow). Brain sections of (C) PRA + ALC, (D) PRA + ALC, and (E) PRA + N-CoQ + ALC, show the cerebellum with almost normal histological features, a well-defined molecular (red arrow), granular (yellow arrow) and Purkinje layers or pyramidal layer (black arrow). ALC, acetyl-L-carnitine; H&E, hematoxylin and eosin; N-CoQ, nano-Coenzyme Q; PRA, propionic acid

enhancement of the bioavailability of the native compound.[13] The current study demonstrated for the first time the potential role of the mono- or combined-therapies of ALC and N-CoQ as neuroprotective agents against PRA toxicity in rats, this was revealed by the decline in serum IL-1β and TNF-α levels and brain NO and lipid peroxide as well as increase brain levels of SOD and GSH, and improvement of the altered expressions of BDNF and P-CREB proteins. Unsurprisingly, combined therapy also improved brain architecture.

5 | CONCLUSION

In conclusion, the combination therapy with ALC or N-CoQ is considered as a useful tool to prevent brain injury induced by PRA. BDNF and CREB protein expressions are involved in both PRA toxicity and treatment.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this study through the Undergraduate Student's Research Support Program (Grant number URSP – 4 – 19 – 19).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

AA suggested the topic and participated in the experimental design and revised the final manuscript. IH codrafted the manuscript and carried out the molecular genetic studies, performed the statistical analysis, and participated in the sequence alignment and drafted the manuscript. BA codrafted the manuscript. MA and KA participated in the sequence alignment and drafted the manuscript. RA and SA acquisition of data and designed the experimental and participated in the biochemical analysis and data collections.

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How to cite this article: Alhusaini A, Hasan IH, Alrumayyan B, et al. Neuroprotective efficacy of nano-CoQ against propionic acid toxicity in rats: Role of BDNF and CREB protein expressions. *J Biochem Mol Toxicol*. 2020;e22449. https://doi.org/10.1002/jbt.22449