Toxicology Reports 10 (2023) 17–26

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Calcium voltage-gated channel subunit alpha 1 C and glial fibrillary acidic protein signaling pathways as a selective biomarker in predicting the efficacy of liposomal loaded co-enzyme Q in the autistic rat model

Doaa M. Elhefnawei [a], Ahlam H. Mahmoud [a], Mai O. Kadry [a,*], Asmaa K. AL-Mokaddem [b], Mohamed A. Badawy [d], Mohamed A. EL-Desouky [c]

[a] Department of Therapeutic Chemistry, National Research Centre, Dokki, Giza 12622, Egypt
[b] Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt
[c] Department of Organic chemistry, Faculty of Science, Cairo University, Egypt
[d] Department of Biochemistry, Faculty of Science, Cairo University, Egypt

ARTICLE INFO

Keywords:
Autism spectrum disorder
Co-enzyme Q10
Liposomal co-enzyme Q10
CACNA1C
GFAP

ABSTRACT

Autism spectrum disorder (ASD) is an extreme neuropsychotic disturbance with both environmental and genetic origins. Sodium propionate (PPA) a metabolic bioproduct of gut microbiota is well-thought-out as a successful autism animal model. Nevertheless, Liposomal drug delivery system possess the advantagous of biocompatibility, targeting organs, ability to carry large drug payloads and skipping macrophages for this purpose the current study was carried out to investigate the hypothesis that Calcium Voltage-Gated channel subunit alpha 1 C (CACNA1C) and glial fibrillary acidic protein (GFAP) signaling pathways crosstalk with the efficacy of Co-enzyme Q10 (Co-Q10) and liposomal loaded Co-enzyme Q10 (L Co-Q10) in PPA mediated autistic rat model. Autism was conducted by buffered PPA (500 mg/Kg b.wt) daily for 5 consecutive days subsequently treatment via Co-Q10 in a dose of (10 mg/kg b.wt) and L Co-Q10 (2 mg/kg b.wt) for four weeks then the autistic model was followed for signs of autism at different time intervals of (one, two and four weeks). The control, PPA intoxicated, and treated groups were subjected to behavioral tests (Y-Maze and open field), antioxidant analysis, gene expression analysis, and histological examination at different time intervals of the study. The results revealed that Co-Q10 and L Co-Q10 significantly elevated antioxidative stress biomarkers, comprising superoxide dismutase (SOD), glutathione (GSH), and total antioxidant capacity (TAC). In addition, they significantly ameliorated the oxidative stress biomarker malondialdehyde (MDA). Meanwhile, they significantly downregulated GFAP and CACNA1C mRNA gene expressions, Co-Q10 and LCo-Q10 showed improvement in almost brain regions post PPA histopathological alterations, even better results were manifested via LCo-Q10 groups. These results showed the superiority of LCo-Q10 over Co-Q10 in competing autism. In conclusion: The administration of anti-inflammatory and antioxidant agents such as Co-Q10 and L Co-Q10 may represent a promising strategy to counteract pathological behaviors in ASD model via targeting organs, increasing retention time, and reducing side effects.

1. Introduction

Autism (ASD) is a neurodevelopmental disturbance categorized via impaired and repreating behaviors, alterated brain development and at last social deficits. ASD pathogenesis is highly genetic in origin with the help of some environmental factors. Several reports deduced that ASDs is contributed to oxidative stress. The brain is susceptiable to oxidative stress contributing to its great aerobic metabolism frequency and energy production. ROS is widely abundant to contribute to some neurobehavioral disorders including autism and schizophrenia. Impairments in working memory and cognitive or behavioral flexibility are commonly reported in many neuropsychiatric disorders such as autism [1].

propionic acid (PPA) is a dietary bioproduct of short-chain fatty acid

* Corresponding author.
E-mail addresses: doaasnz@gmail.com (D.M. Elhefnawei), ahlam@hotmail.co.uk (A.H. Mahmoud), maiosman666@yahoo.com (M.O. Kadry), asmaa.khairy@gmail.com (A.K. AL-Mokaddem), mabadawy52@gmail.com (M.A. Badawy), meldesouky@sci.cu.edu.eg (M.A. EL-Desouky).

https://doi.org/10.1016/j.toxrep.2022.12.003
Received 21 September 2022; Received in revised form 29 November 2022; Accepted 6 December 2022
Available online 7 December 2022

widely used as food preservative and metabolic bioproduct of intestinal microbiota in gut. Nevertheless, highly concentrating in the gut, it is able cross the BBB, cross cell membranes, and concentrate in cells, contributing to its acidification. This process can modify neurotransmitter secreation, thus influencings neuronal communication and behavior leading to developmental delay. Furthermore, PPA can alter genes, related to autism pathogenesis, that controls neuroplasticity, oxidative stress, neurotransmitters, neurodevelopment, mitochondrial function and inflammation [2]. PPA can induce brain and behavioral disturbance in animal model identical to those of autistic human beings, via fluctuating brain FA metabolism [3].

GFAP is a key hallmark intermediate filament protein of astroglial activation marker and reactive gliosis in response to neurodegeneration. Reactive gliosis is essential for the protective role of astrocytes; the main type of glial cells in the central nervous system (CNS) at acute stages of injury, Overexpressed GFAP is highly related to astroglial putative cell protection regarding residual neurons. Moreover, GFAP was widely related to regenerative responses and neural plasticity in healthy and injured brain. GFAP protein is accountable for maintaining mechanical strength of the cytoskeleton structure of glial cells and reinforcing the BBB and neighboring neurons. It has been studied in many neurological diseases,including amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease and autism [4].

Calcium channels facilitate Ca2 + influx inside the cell upon membrane polarization. CACNA1C encodes the pore-forming alpha 1 C subunit of the L-type voltage-gated Ca2 + channel (VGCC) Cav1.2, which elevates Ca2 + concentration upon extreme depolarization. Ca2 +channel has a vital role in synaptic efficacy, neuronal firing, neuronal survival and excitation-transcription coupling. Moreover, it modulates GABA transmitting interneuron function. Thus, CACNA1C can influence inter-regional connectivity and brain regional activation [5].

Natural antioxidants have attracted researchers interest all over the past decades among them Co-enzyme Q10, a benzoquinone lipid-soluble agent is abundant in animals, plants, and tissues. Co-Q10 is profound in kidney, muscles, heart, and brain contributing to their elevated energy requirements. Inside the mitochondria and through metabolism, Co-Q10 was utilized as an electron carrier for ATP production and oxidative phosphorylation in the electron transport chain. Outside mitochondria, Co-Q10 is a violent antioxidant, protect cells from free radicals beside the various antioxidants, that hinder ROS release that contributes to oxidative stress regarding to their detrimental influence on proteins, DNA, lipids and mitochondria dysfunction. Co-Q10 protects the intracellular membranes and the cell membrane via shielding the membranes phospholipids from peroxidation. In addition, Co-Q10 can increase the production of key antioxidants. Moreover, Co-Q10 has been shown to have antiapoptotic and inflammatory activities. The potential therapeutic effect of Co-Q10 was reported in oxidative challenge-related disorders and mitochondrial dysfunction diseases as neurodegenerative disorders, diabetes, cancer and cardiovascular disease [6].

Co-enzyme Q10 is highly unsteady and problematic to store a long time contributing to its photosenstivity, extreme hydrophobicity, high molecular weight and thermolability, leading in low bioavailability in vivo post peroral administration. Nevertheless, to enhance the bioavailability and stability via novel pharmaceutical biotechnologies, novel formulations were explored including the nanomedicines, nanoliposomes and solid dispersion system with long-circulating materials that prolonged circulation time, enhance stability, bioavailability, target the brain and cross the BBB [7].

The present study aimed to identify the efficacy of Co-Q10 and LCo-Q10 on ASD-like animal models induced via PPA by investigating GFAP and CACNA1C pathophysiological signaling and the neurobehavioral features following the change in brain structure.

2. Materials and methods

2.1. Chemicals

Sodium propionate, Co-Q10, and LCo-Q10 were purchased from Alfa Aesar (Thermo Fisher (Kandel) GmbH. Erlenbachweg 2.76870 Kandel, Germany), Amoun Pharmaceutical Company S.A.E (1st industrial zone-block 13015 EL Obour city-Cairo, Egypt) and Empirical Labs (1501 Academy Ct. Suite 5, Ft Collins, CO 80524), respectively. Kits for biochemical parameters investigation were obtained from Randox Company (Antrim, UK). RT-PCR kits and primers of CACNA1*C* and GFAP were obtained from Qiagen Company (Germantown, USA). The rest of chemicals are of the maximum analytical grade.

2.2. PPA preparation

Sodium propionate was dissolved in 0.1 M PBS and administered subcutaneously in a dose of 500 mg/kg, pH 7.4) once a day for five consecutive days. This dose was selected based on previous studies [8]. Rats in the control group were injected with saline. In addition, body weight and ASD phenotypes were counted every week.

2.3. The experimental animals

A total of 100 healthy male western albino rats weighing 80–100 gm approximately 21 days old, were obtained from the Animal House of the National research center and used in this study. All experimental procedures for evaluating ASD development were performed on 3-week-old animals. The rats were randomly divided into either control or PPA-intoxicated (ASD) groups. Animals were allowed access to standard rodent chow (Hyochang Science) and tap water ad libitum. All procedures relating to animal care and treatments strictly adhered to the ethical procedures and policies approved by the Animal Care and Use Committee of Cairo University (CUIF1319), National Research Center (19011) and complied with the Guide for Care and Use of laboratory published by the US National Institute of Health. All rats were housed two per cage under controlled environmental conditions (22 ± 1°C) and an established light: dark photoperiod (12:12 hr; lights on 07:00).

2.4. Experimental design

Post one week of acclimatization, animals were randomly divided into ten groups (each of 10 animals) and divided according to the following schedule:

Group1: Animals received phosphate-buffered saline (PBS) and served as a normal control group.

Group 2: Sodium propionate intoxicated group rats received (500 mg/kg body weight) subcutaneously daily for 5 days, in order to induce autistic features and were sacrified one week post induction [8].

Group3: Sodium propionate intoxicated group treated with co-enzyme Q in a dose of (10 mg/kg body weight) intraperitoneally daily for one week [9].

Group 4: Sodium propionate intoxicated group treated with liposomal coenzyme Q in a dose of (2 mg/kg body weight) intraperitoneally daily for one week [9].

Group 5: Sodium propionate intoxicated group received (500 mg/kg body weight) subcutaneously daily for 5 days, and were sacrified two weeks post induction [8].

Group 6: Sodium propionate intoxicated group treated with coenzyme Q in a dose of (10 mg/kg body weight) intraperitoneally daily for two weeks [9].

Group 7: Sodium propionate intoxicated group treated with liposomal coenzyme- Q in a dose of (2 mg/kg body weight) intraperitoneally daily for two weeks [9].

Group8: Sodium propionate intoxicated group received (500 mg/kg body weight) subcutaneously daily for 5 days, and were sacrified post

four weeks of induction [8].

Group 9: Sodium propionate intoxicated group treated with coenzyme- Q in a dose of (10 mg/kg body weight) intraperitoneally for four weeks [9].

Group 10: Sodium propionate intoxicated group treated with liposomal coenzyme- Q in a dose of (2 mg/kg body weight) intraperitoneally for four weeks [9].

2.5. Blood sampling and brain tissue preparation

Finally, rats weight was recorded and then anesthetized with carbon dioxide, blood was gathered from retrorbital vein. Separation of serum via centrifugation at 5000 rpm for 15 min and then stored at − 80 ∘C for further estimation of ALT, AST and glutathione activities. Animals has been sacrificed via cervical dislocation and further brain tissues were carefully separated and weighed, and then was homogenized in 4 volumes of phosphate buffer, pH 7.4, using a Teflon homogenizer (Glass-Col homogenizer, Terre Haute, USA). whereas the second portion was kept in 10% formaldehyde, for further histopathological investigations.

2.6. Neurobehavioral testing

2.6.1. Y- Maze test

The Y-maze was utilized as an indicator of general locomotor activity, short-term memory and stereotypic behavior. Consequently, extemporaneous variation was investigated via Y-maze that contains three uniformaly spaced arms (130°, 42 cm long, and 20 cm high). The floor was manufactured from Perspex and is 6 cm in width. Indvidual rat was placed in one of the arm compartments and was allowed to freely move. The sequence of arm entries is manually recorded. An alternation is defined as entry into all three arms consecutively, The number of maximum spontaneous alternations is then the total number of arms entered minus two and the percentage alternation is calculated as {(actual alternations /maximum alternations) x 100}. For each animal, the Y-maze testing was carried out for 5 min [10].

2.6.2. Open field test

This test is performed to assess locomotor activity in rodents. The open-field test arena is a square box (50 ×50 cm) divided into 25 squares of identical size (10 ×10 cm). The experimental rat was placed in the center area of the arena and allowed to explore the arena for five minutes. The total distance that moved (total bar crossing in units) in the whole arena was measured. The overall distance that each rat moved is considered an indicator of locomotor activity [11].

2.6.3. Measured parameters

2.6.3.1. Serum aspartate and alanine aminotransferases. The Randox Company offered spectrophotometric kits that were used to estimate the ALT and AST activity. In a nutshell, pyruvate and L-glutamate are produced when L-alanine interacts with oxoglutarate in the presence of ALT. On the other hand, L-aspartate transforms into oxaloacetate and L-glutamate when AST is present. The hydrazone derivative, which can be detected at 540 nm, is produced when the generated pyruvate or oxaloacetate combines with 2,4-dinitrophenyl hydrazine in an alkaline solution. The Randox Company offered spectrophotometric kits that were used to estimate the ALT and AST activity. In a nutshell, pyruvate and L-glutamate are produced when L-alanine interacts with oxoglutarate in the presence of ALT. On the other hand, L-aspartate transforms into oxaloacetate and L-glutamate when AST is present. The generated pyruvate or oxaloacetate interacts with 2,4-dinitrophenyl hydrazine in an alkaline solution to produce the hydrazone derivative [12].

2.6.3.2. Brain malondialdehyde level. Randox Company kits were utilized to estimate MDA [13].

2.6.3.3. Brain superoxide dismutase. Using Randox Company-provided commercially accessible kits, SOD was calculated spectrophotometrically. The assay depends on the enzyme's capacity to prevent phenazine methosulphate from mediating the dye's reduction [14].

2.6.3.4. Brain Total antioxidant capacity. TAC was calculated spectrophotometrically using kits that are available commercially and were provided by the Randox Company. The response of antioxidants in the sample with a predetermined amount of exogenously supplied hydrogen peroxide is used to determine the antioxidative ability (H2O2) A portion of the supplied hydrogen peroxide is removed by the antioxidants in the sample. Through an enzymatic procedure that involves the creation of a colored product from 3,5,dichloro-2-hydroxy benzensulphonate, the remaining H2O2 is measured colorimetrically [15].

2.6.3.5. Serum glutathione level. Glutathione level was investigated utilizing kits purchased from Randox company [16].

2.6.3.6. Quantitative real-time-polymerase chain reaction (qRT-PCR) for analysis of brain CACNA1C and GFAP mRNA gene expression. Using a Tissue Ruptor and QIAzol lysis reagent, the brain tissues were homogenised (Roche). Following the manufacturer's instructions, Tripure Isolation Reagent (Roche) was used to isolate total RNA. According to the manufacturer's instructions, complementary DNA (cDNA) was produced using Superscript Choice Systems (Life Technologies, Breda, Netherlands). Quantitative real-time PCR was carried out using SYBR green PCR Master Mix (Applied Biosystems, CA, USA) in accordance with the manufacturer's instructions to evaluate the mRNA expression of CACNA1C and GFAP. In a 25xl reaction volume, 200 ng of each primer and 12.5xl of 2 SYBR green Master Mix were combined with 5 xl of cDNA. Table 1 provides a description of the primer sequences. Following is the temperature profile: 94 °C for 3 min, 94 °C for 20 s, and 73∘C for 35 s for 40 cycles [17]. Primers used in this study were mentioned in Table 1.

2.6.3.7. Histological examination. Rats brain were dissected out and kept at 10% neutral buffered formalin for fixation. Brain tissue samples were processed and embedded in paraffin wax. 5 μm sections were cut and stained with hematoxylin and eosin (H&E) [18]. Stained tissue sections were examined using an Olympus microscope (BX43, Olympus, Japan).

2.6.3.8. Statistical analysis. Data were expressed as means ± SEM. Statistical analysis was performed using the Instat-3 computer program (Graph pad software Inc, San Diego, CA, USA). One way analysis of variance (ANOVA) by SPSS 12 program followed by Post HOC test was used to determine the differences between means of different groups. The level of significance was set at $P < 0.05$ using Tukey's test.

Table 1
Primers sequence.

Primer name	Primer sequence 5′….3′
CACNA1C	Forward primer, 5 '- GCTCGGATCTCATCCCTCTC-3′ Reverse Primer, 5 '-GACGCATCTGAGCACGGA- 3′
Glial fibrillary acidic protein (GFAP)	Forward primer, 5 '-TACAGACAGGAGGCGGATGAAGCC- 3′ Reverse Primer, 5 '-GCATTTGCCTCTCCAAGGACTC-3′
β- actin	Forward primer, 5 '-CCCATCTATGAGGGTTACGC- 3′ Reverse Primer, 5 '-TTTAATGTCACGCACGATTTC- 3′

3. Results

3.1. Modulation of behavioral test

3.1.1. Y- Maze test

Propionic acid intoxication in the three time intervals of (1, 2 and 4 weeks) deduced a significant reduction in Y- maze test as compared with the control value reflecting autism induction, with the fourth week showing the most significant reduction. Meanwhile,treatment with Co-Q10 and LCo-Q10 revealed a significant elevation in Y- maze test as compared with PPA groups, with LCo-Q10 in the fourth week showing the most significant impact (Table 2).

3.1.2. Open- field test

The result of the open-field test showed that PPA intoxicated rats traveled significantly less total distance (number of squares crossed) compared to those traveled by rats of control, indicated that rats of the PPA group were significantly less active during the three time periods. On the other hand, rats of the Co-Q10 and LCo-Q10 treated groups traveled a far greater distance than those of the PPA groups, they were significantly more active especially with LCo-Q10 during the fourth week (Table 2).

3.1.3. Modulation of liver function

In this study serum AST and ALT activities during all time intervals (1, 2 and 4 weeks) were significantly higher post PPA intoxication in comparison with that of the normal value, PPA intoxicated rats in the fourth week revealed the most significant elevation in AST activity however, the most significant increment in ALT activity was in the first week. On the other hand, treatment with Co-Q10 and LCo-Q10 significantly reduced AST and ALT activities at all time intervales in comparison with that of PPA intoxicated rats with LCo-Q10 in the fourth week reflecting the most significant effect (Table 3).

3.1.4. Modulation of oxidative stress biomarkers

3.1.4.1. Brain malondialdehyde level. MDA level was significantly higher in PPA intoxicated groups than in the controls during the first, second and fourth week, in contrast, Recorded values in (Table 3) estimated that there was a significant diminution in MDA levels post Co-Q10 and LCo-Q10 treatment throughout all epochs, at the first and second weeks, there was a more significant reduction with the treatment of LCo-Q10 than Co-Q10 but at the fourth week, Co-Q10 is more effective than LCo-Q10.

3.1.4.2. Brain SOD and TAC levels. PPA intoxication induced a state of oxidative stress evidenced by a significant reduction in SOD activity and TAC level in brain tissue as compared with the control value during all time intervals (Table3). PPA in the fourth week revealed the most significant reduction in both SOD activity and TAC level as compared with the control value. On the other hand, Treatment with Co-Q10 and LCo-Q10 revealed a significant increase in SOD activity and TAC level in all time intervals as compared with PPA intoxicated rats. Furthermore, LCo-Q10 treatment at the fourth week showed the most significant influence.

3.1.4.3. Serum glutathione level. The serum level of GSH from both intoxicated and treated groups is illustrated in (Table3). As represented, PPA intoxicated rats showed a significant reduction as compared with the control value through all periods, there were tendencies for increased GSH levels in both treated Co-Q10 and LCo-Q10 groups over all periods of time, the fourth week highlighted the most significant improvement in GSH level post treatment with Co-Q10.

3.1.4.4. Impact of PPA on mRNA gene expression of GFAP and CACN1C. The data presented in (Fig. 1a, b) revealed that PPA intoxication upregulated the mRNA gene expressions of GFAP and CACN1C, specifically in the fourth week as compared to the control value. Administration of Co-Q10 and LCo-Q10 noticeably down-regulated their expression levels during all time intervals, with the LCo-Q10 regimen at the fourth week showing the most significant reduction in both GFAP and CACN1C gene expressions, and mainly reached the normal value as compared to the PPA group.

3.1.4.5. Histological examination results. As illustrated in (Fig. 2), histopathology of brain sections from group 1 revealed the normal histological structure of different brain regions of the brain. Different regions of the hippocampus, including CA1, CA2, CA3, CA4, and dentate gyrus (DG) were normal. The cerebral cortex and cerebellum were normal as well. On the contrary, the group 2 showed some histopathological alterations; in the hippocampus, few degenerating cells were observed in CA1 and CA3 regions, while CA2, CA4, and DG showed numerous dark eosinophilic neurons. Cerebral cortex exhibited some dead cells as well as Purkinje cell necrosis in the cerebellum. Mild improvement was detected in the group 3 as different regions of the hippocampus showed the existence of dark degenerating neurons that were especially increased in CA3, CA4, and DG. Some eosinophilic dead neurons were detected in the cerebral cortex presence of some necrotic Purkinje cells in the cerebellum. Moderate improvement was noticed in group 4 as only a few degenerating cells were detected in CA2, CA3, and CA4 regions of the hippocampus as well as the existence of some degenerating cells in the cerebral cortex and apparently normal cerebellum.

After 2 weeks (Fig. 3), group 5 showed more prominent changes; all regions of the hippocampus showed numerous dark degenerating neurons with the presence of microglia and marked loss in cell density, especially in CA3 and CA4 regions of the hippocampus. The cerebral cortex exhibited neuronal degeneration with neuronophagia. Increasing numbers of dead Purkinje cells were noticed in the cerebellum. Brains of group 6 showed moderate improvement, only a few dead cells were detected in the CA1 region, meanwhile, an apparently normal CA2 and CA3 regions were seen. Few dark cells were observed in DG. The cerebral cortex was apparently normal. Few necrotic Purkinje cells were detected in the cerebellum. Group 7 showed apparently normal brain regions without any detectable alterations.

After 4 weeks (Fig. 4). Group 8 showed marked histopathological changes represented by diffuse neuronal necrosis in all regions of the

Table 2
Impact of different time intervals of Co-Q10 and LCo-Q10 treatment on behavioral tests (Y-maze and open field).

Groups Parameters	Control (zero time)	PPA (1 Week)	Co-Q10 (1Week)	LCo-Q10 (1Week)	PPA (2Weeks)	Co-Q10 (2Weeks)	LCo-Q10 (2Weeks)	PPA (4Weeks)	Co-Q10 (4Weeks)	LCo-Q10 (4Weeks)
Y-maze result	93.26 ±	61.36 ±	69.76 ±	75 ±	53.33 ±	90.3 ±	82.91 ±	39.52 ±	77.77 ±	88.88 ±
% improvement	3.42[a]	3.39[b]	3.09[b] 9%	14.43[b] 14.63%	3.33[b]	5.78[a] 39.64[%]	9.13[c] 31.72%	5.26[d]	14.69[e] 41%	6.41[a] 52.93%
open field result	57.33 ±	20.66 ±	22.33 ±	25 ± 4.04[b]	15.66 ±	30.66 ±	34 ± 1.15[d]	13 ± .0[c]	34 ± 3.05[e]	40.66 ±
% improvement	2.4[a]	4.7[b]	1.45[b] 2.91%	7.57%	8.56[c]	3.84[d] 26.16%	31.99%		36.63%	2.33[d] 48.25%

Data are expressed as Mean± SE at $P \leq 0.05$ by one way ANOVA test where (n = 10). Similar letters are not significantly different from each other meanwhile different letters are significantly different from each other.

Table 3
Influence of different time intervals of Co-Q10 and LCo-Q10 treatment on biochemical parameters of PPA intoxicated rats.

Groups Parameters	Control (zero time)	PPA (1 Week)	Co-Q10 (1Week)	LCo-Q10 (1Week)	PPA (2Weeks)	Co-Q10 (2Weeks)	LCo-Q10 (2Weeks)	PPA (4 Weeks)	Co-Q10 (4Weeks)	LCo-Q10 (4Weeks)
AST (mmol/ml)	54.78 ±	79.38 ±	66.6 ±	59.87 ±	105.94 ±	97.32 ±	85.52 ±	107.5 ±	70.73 ±	68.53 ±
%	7.21[a]	7.78[b]	1.13[c]	7.73[d]	9.67[e]	1.13 [f]	10.34 [g]	9.64[e]	0.21 [h]	0.24 [h]
improvement			23.33%	35.62%		15.74%	37.28%		67.12%	71.14%
ALT(mmol/ml)	14.25 ±	128.52 ±	109.39 ±	109.12 ±	122.88 ±	22.63 ±	20.06 ±	115.02 ±	24.1 ±	19.64 ±
%	0.12[a]	1.06[b]	0.25[c]	0.51[c]	3.98[d]	3.12[e]	3.68 [f]	0.15 [g]	0.57[e]	3.73 [f]
improvement			134.25%	136.14%		703.5%	721.54%		638%	669.33%
MDA	12.3 ±	20.54 ±	11.5 ±	8.9 ±	19.99 ±	7.9 ±	6.42 ±	12.64 ±	5.3 ±	8.5 ±
%	0.13[a]	0.25[b]	0.19[c]	0.18[d]	0.15[b]	0.21[d]	0.11[e]	0.19[a]	0.13[e]	0.14[d]
improvement			73.49%	94.63%		98.29%	110.33%		59.67%	33.66%
SOD	91.1 ±	76.42 ±	80.63 ±	81.68 ±	59.27 ±	89.78 ±	89.96 ±	44.16 ±	86.78 ±	90.09 ±
%	2.3[a]	3.8[b]	6.1[c]	1.92[c]	2.9[d]	3.3[a]	3.2[a]	2.8[e]	1.9[f]	5.4[a]
improvement			4.62%	5.77%		33.49%	33.69%		46.78%	50.42%
TAC	2.1 ±	0.42 ±	0.63 ±	0.68 ±	0.27 ±	0.78 ±	1.36 ±	0.16 ±	1.78 ±	2.09 ±
%	0.14[a]	0.1 3[b]	0.01[c]	0.02[c]	0.01[d]	0.012[c]	0.023[e]	0.015[f]	0.12[g]	0.02[a]
improvement			10%	12.38%		24.29%	51.9%		77.14%	91.9%
GSH (μg/ml)	0.08 ±	0.04 ±	0.06 ±	0.08 ±	0.01 ±	0.12 ±	0.11 ±	0.04 ±	0.21 ±	0.13 ±
%	0.0012[a]	0.00809[b]	0.00233[c]	0.01146[a]	0.00088[d]	0.02088[e]	0.01084[e]	0.02484[b]	0.03543[f]	0.00328[e]
improvement			25%	50%		137.5%	125%		212.5%	112.5%

Data are expressed as Mean± SE at $P \leq 0.05$ by one way ANOVA test where (n = 10). Similar letters are not significantly different from each other meanwhile different letters are significantly different from each other.

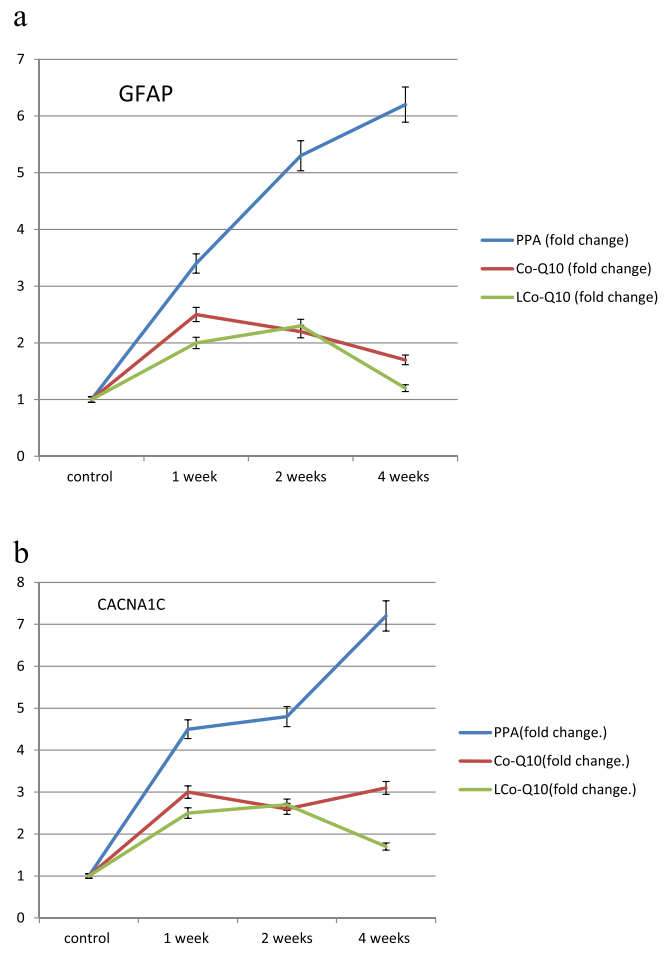

Fig. 1. **a:** Impact of different time intervals of Co-Q10 and LCo-Q10 treatment on brain GFAP mRNA gene expressions in PPA intoxicated rats. Data are expressed as fold change ± SE at $P \leq 0.05$ by one way ANOVA test where (n = 10). **b:** Impact of different time intervals of Co-Q10 and LCo-Q10 treatment on brain CACNA1C mRNA gene expressions in PPA intoxicated rats. Data are expressed as fold change ± SE at $P \leq 0.05$ by one way ANOVA test where (n = 10).

hippocampus marked cell loss. DG in some of the examined sections showed hemorrhage. The cerebral cortex exhibited numerous dark degenerating neurons and the cerebellum showed diffuse necrosis in Purkinje cells. Marked improvement was noticed in group 9 that showed apparently normal hippocampus except for a few degenerating cells in the CA1 region. Both cerebral cortex and cerebellum were apparently normal. Even better results were seen in group 10 as all brain regions were apparently normal. A summary of the detected histopathological alterations and their severity in different brain regions is shown in (Table 4).

4. Discussion

Propionic acid, a short-chain fatty acid, can enter the brain and influence a variety of physiological processes. PPA can affect the intracellular pH, cell signaling, production and release of neurotransmitters, mitochondrial function, inflammation, oxidative stress, lipid/mitochondrial metabolism, immunological function, gap junction gating, gene expression regulation and promote intracellular calcium release, all of which can potentially affect neuronal communication and behavior. The behavioral and physiological findings seen in the PPA animal model and ASD may be explained by several of these effects, which resemble many of the pathways linked to ASD [19].

According to the current data, systematic PPA intoxication significantly decreased the spontaneous alteration in the Y-maze behavioral test, whereas Co-Q10 and LCo-Q10 treatment significantly improved these abnormal behavioral test. Co-Q10's antioxidant activity may be the cause of the ameliorative effect of Co-Q10 and LCo-Q10 on the animal performance in a Y maze exercise. This effect is due to Co-Q10 ability to regenerate other endogenous antioxidants as vitamin C & E. Additionally, they boosted their levels in the mitochondria of rat brain cells, which improved memory and learning functions by lowering the synthesis of protein carbonyls and oxidative stress in the brain.

The level of LCo-Q10 was observed in various rat brain regions and proved the bioavailability of the LCo-Q10 to selected brain regions after its administration into the right brain ventricle. Administration of LCo-Q10 may create the basis for modulation of neuronal activities in specific brain regions [20].

The impact of PPA was consistent with other research [21] that showed short-term memory loss and a typical behavior as a result of its oxidative stress on the neuronal cells. Co-Q10 ability to replenish endogenous antioxidants like vitamins C and E is the cause of the Co-Q10 and L Co-Q10 impact. The current findings corroborated several

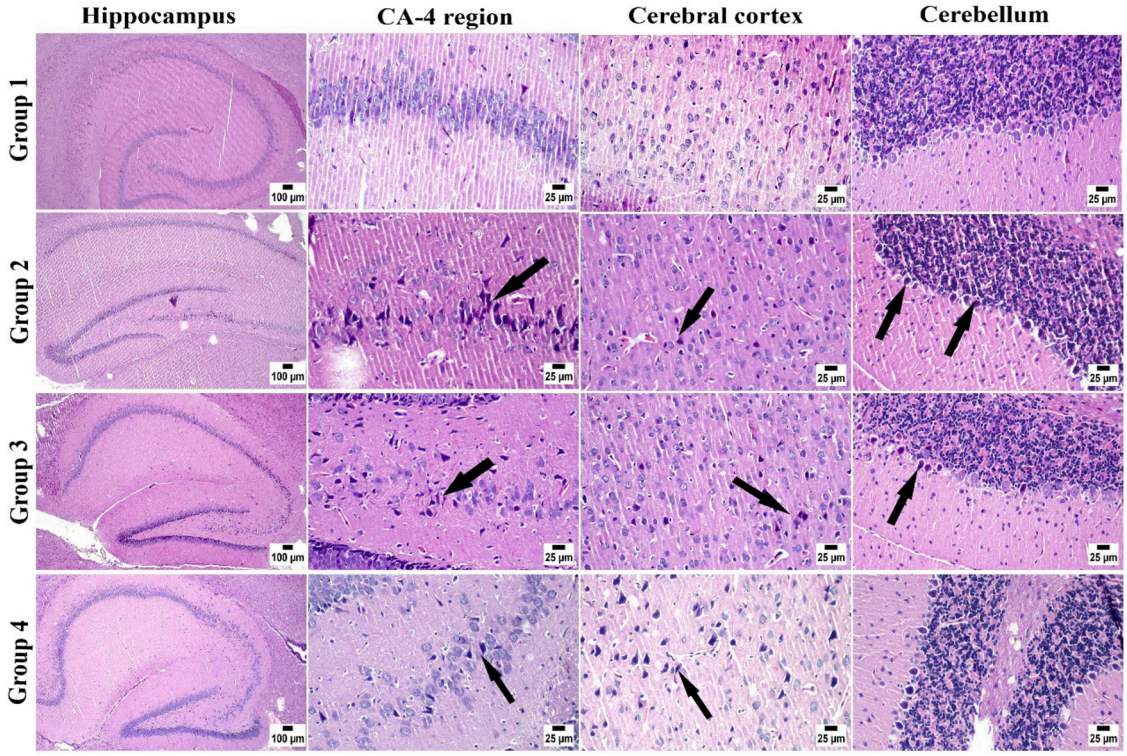

Fig. 2. Photomicrographs of brain (H&E stained) showing different brain regions in the experimental groups, (black arrows) indicate degenerating neurons in different brain regions.

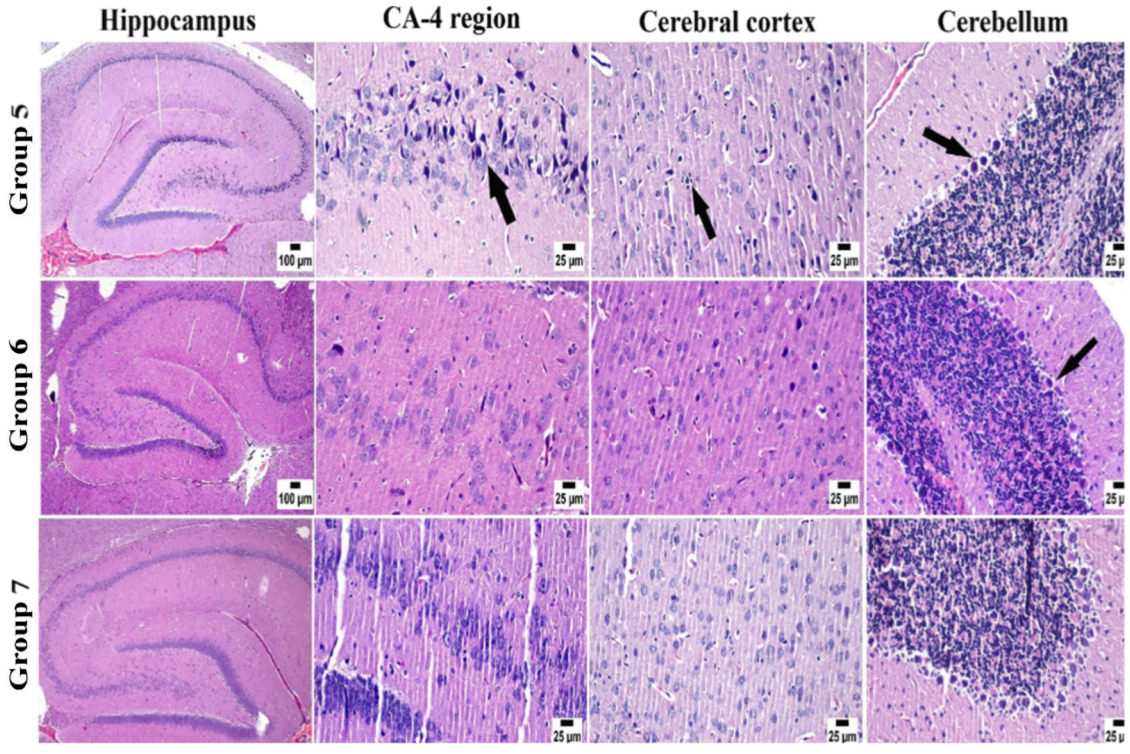

Fig. 3. Photomicrographs of brain (H&E stained) showing different brain regions in the experimental groups, (black arrows) indicate degenerating neurons in different brain regions.

prior research hypotheses regarding the antioxidant role of Co-Q10. Due to low solubility and reduced bioavilability of Co-Q10 liposomal loaded nanomedicines were invented to overcome the obstacle of poor solubility, enhance bioavilability, retension time target organs and skip macrophage engulfing and cross the BBB [22].

The open field test was performed to evaluate the rats' general

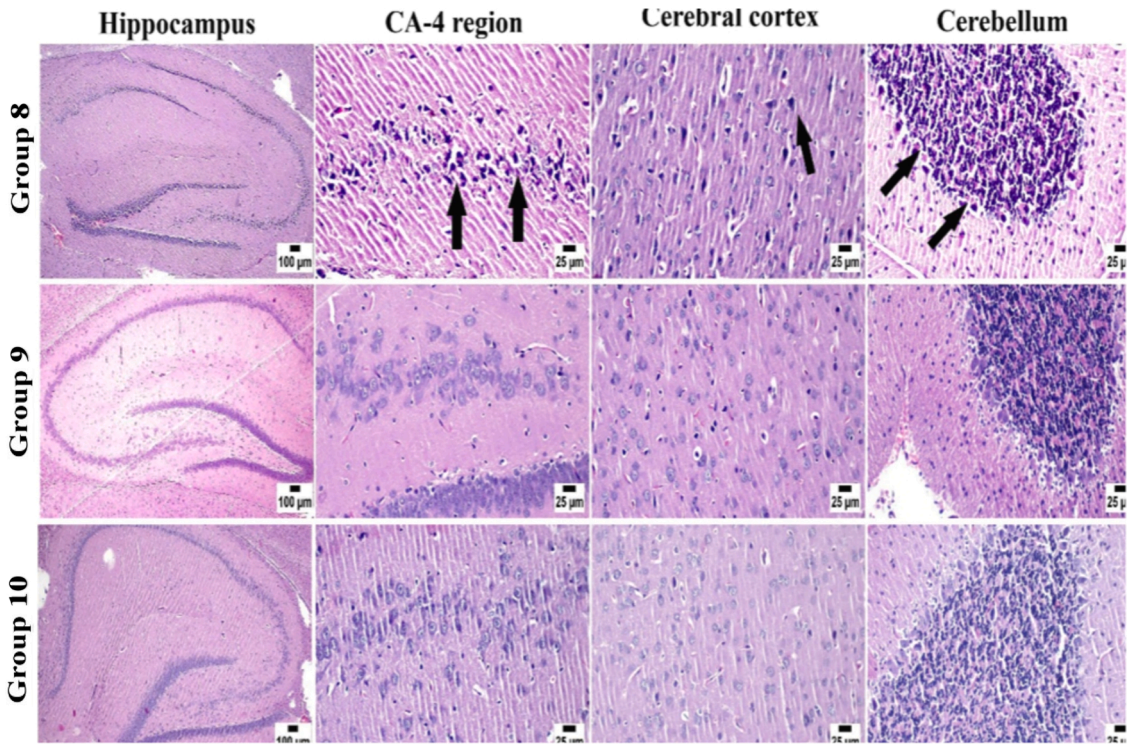

Fig. 4. Photomicrographs of brain (H&E stained) showing different brain regions in the experimental groups, (black arrows) indicate degenerating neurons in different brain regions.

Table 4
Qualitative score of the detected histopathological alterations in brain of rats from different experimental groups.

	Groups	Degenerating neurons within Hippocampus	Degenerating neurons within Cerebral cortex	Degenerating neurons within Cerebellum
1	Normal	–	–	–
2	PPA (1 week)	+ +	+ +	+ +
3	Co-Q10 (1 week)	+ +	+	+
4	LCo-Q10 (1 week)	+	–	–
5	PPA (2weeks)	+ ++	+ ++	+ ++
6	Co-Q10 (2 weeks)	+	–	+
7	LCo-Q10 (2 weeks)	–	–	–
8	PPA (4 weeks)	+ ++ +	+ ++ +	+ ++ +
9	Co-Q10 (4 weeks)	+	–	–
10	LCo-Q10 (4 weeks)	–	–	–

(-) absent, (+) few, (++) moderate, (+++) Numerous and (++++) Numerous and diffuse abnormalities.

locomotor and exploratory behavior. In open field test (Table 2), PPA rats showed significantly reduced locomotor activity, compared with those of controls, Co-Q10 and LCo-Q10 treated rats due to a neurotoxic and neuroinflammatory responses in animals. Motor skills and behaviors develop in the body and brain of newborn rats during the first three weeks of life, intoxication via PPA in this stage, manifest abnormal behavioral responses, inflammation, oxidative stress, and aberrant neurotransmission, appeared in their brains, were reflected by locomotor activity impairment. Additionally, based on the current histological abnormalities, cerebellar Purkinje cell necrosis may contribute to the motor activity impairments.

According to previous researches, reduced exploratory behavior and constricted locomotor activity, represented an unfamiliar environment due to immune system stimulation. [23] supported the current aberrant cerebellum histology finding where, the patients with ASD have been shown to have a cerebellum with an active and chronic neuroinflammatory process responsible for the motor dysfunctions.

Accordingly, Co-Q10 treatment during the open field test could be the result of psychomotor stimulation or reduced anxiety. Similarly, it has been proposed that Co-Q10 supplements; which influence mitochondrial metabolism, could alleviate depression via chelating ROS and reducing oxidative stress [24].

The current findings showed that PPA-treated rats had higher activities of serum AST and ALT than control rats. However, treatment with Co-Q10 and LCo-Q10 considerably decreased their levels. Increasing acidity by PPA made oxidative stress, mitochondria dysfunction, and hepato-necrosis leading to a decrease in Co-Q10 concentration. Mitochondrial dysfunction causes the tricarboxylic acid cycle (TCA) abnormality and loss of cellular integrity of some organs such as liver and enzymes of this organ come out of the cell resulting in cellular leakage and an increase in serum levels. The significantly higher level of serum AST and ALT in the PPA autistic rat model were consistent with other studies [25].

In the current study, serum AST and ALT activities were significantly normalized post Co-Q10 and LCo-Q10 treatment. Co-Q10 possesses a strong antioxidant efficacy that can protect cells from ROS, lipid peroxidation, inflammation and apoptotic activities brought on by harmful substances. Due to long-circulating period, improved stability, prolonged circulation and increased bioavailability, LCo-Q10 therapy had the most notable impact in this aspect. LCo-Q10 can mitigate hepatotoxicity by altering the amounts of the proteins. Additionally, LCo-Q10 is a candidate drug to be utilized for patients who are inebriated due to the liposome's capacity to stick to the cell membrane and release its content inside the cell. This gives it superior effects over its non

liposomal analogue. The outcomes are consistent with other investigations of [26].

The present data revealed that intoxication with PPA caused a significant elevation in MDA and reduction in SOD, TAC and GSH due to excessive free radicals production. The supplementation of Co-Q10 and LCo-Q10 ameliorate this devision via their powerful antioxidant effect and free radical scavengering effect.

The brain is easily affected via oxidative damage contributing to its high lipid concentration, high oxidative metabolic rate, and low intracellular antioxidant system. PPA induces polyunsaturated fatty acid oxidation, which resulted in MDA increase and biomolecular deterioration in the current study. From this deterioration, reduction in GSH, SOD and consequently TAC.

Glutathione is the first soldier to encounter ROS. Therefore, an increase in its consumption leads to a decrease in its concentration, inhibition of glutathione reductase, and a decrease in defense mechanisms against ROS. This will be reflected on the brain cells causing damage, affect the behavior of the animal and increase the side effects of autism [27].

Previous researches have shown that children with ASD showed increase in MDA levels than healthy control groups due to oxidative stress, and it has been hypothesized that this may be due to their antioxidant mechanisms are still immature [28]. All these deteriorations were alleviated by Co-Q10 or LCo-Q10 supplementation. They may increase gene expression of antioxidant enzymes, consequently improving the TAC levels. Several studies showed the protective effects of Co-Q10 supplement which is similar to this finding [29].

Generally, The neuroprotective efficacy of Co-Q10 has been demonstrated by suppressing mitochondrial dysfunction and oxidative stress as shown in disease models of cognitive dysfunction [30].

The supplementation may have an influence on these oxidative stress biomarkers through a variety of direct and indirect pathways**.** From a direct perspective, Co-Q10 preserves the regular electron transport in the mitochondrial electron transport chain (METC), resulting in a decrease in the production of superoxide (O2 •). In addition, investigations conducted both in vivo and in vitro have shown that Co-Q10 administration may reduce lipid peroxidation. From an indirect perspective, Co-Q10 can regenerate tocopherol, the reduced form of vitamin E's active substance, by converting the product of the reaction between vitamin E and lipid peroxidative free radicals. Furthermore, Co-Q10 could eliminate the effects of oxidative stress on the gene aspect by stimulating nuclear factor erythroid 2-related factor 2 (Nrf-2), a transcription factor that regulates cellular responses to oxidative stress by regulating a number of ROS-detoxifying enzymes [31].

In the current study, the PPA autistic model exhibited a significantl up regulation in brain GFAP and CACNA1C gene expressions as compared with typical development controls. However, administration of CO-Q10 and LCO-Q10 markedly down regulated these gene expressions.

Neuroepithelial progenitor cells (NPCs), which are produced by neural stem cells, develop into neuronal or glial cells. Oligodendrocytes and astrocytes, two types of glial cells, participate in the growth, connection, and defence of neurons. Reactive glial cells multiply and release fibrillary acidic protein (GFAP) during traumatic brain injury to prevent defective axonal regrowth, which results in gliosis. Additionally, to remove poisons and injured cells, glial and microglial cells release inflammatory cytokines, which leads to neuro-inflammation. Because it eliminates injured cells and prevents damaged axons from regrowing, some researchers view gliosis as a protective process. However, it is safe to assume that gliosis will significantly influence neuronal architecture and connections if it happens during the earliest phases of brain development. Cortical, limbic, and cerebellar regions of the ASD brain were shown to have abnormal neural connectivity and higher regional cell densities. According to supporting data, glial cell dysfunction may be the root cause of ASD. Particularly, it was discovered that, in contrast to age-matched healthy controls, GFAP was abundantly expressed in the ASD brain [32].

Glia exhibit striking morphological and gene expression changes in neurodegenerative diseases; reactive astrocytes proliferate, especially in the vicinity of amyloid plaques, and glial fibrillary acidic protein (GFAP) expression is elevated. Additionally, reactive astrocytes increased intercellular Ca^{2+} waves, Ca^{2+} transient frequency, and resting Ca^{2+} levels. These factors could all increase the release of different gliotransmitters like glutamate, D-serine, ATP, and GABA [33]. This suggests a strong correlation between the levels of GFAP and CACNA1C gene expressions.

In relation to GFAP, the rise in expression is brought on by PPA-induced neuronal damage and astrocyte activation. Astrocytes were found to produce more GFAP and other neurotoxic substances in response to any threat to the nervous system. Following neurodegeneration, this acts as a compensatory mechanism.

The outcome agrees with earlier research of [34] which revealed that serum GFAP levels were noticeably higher in kids with ASD and that their expression was related to the severity of autism in the Chinese population. They proposed using serum GFAP concentrations as an additional diagnostic tool for the early identification of ASD. After traumatic brain injury, elevated GFAP levels have been observed in the CNS, as well as serum.

Co-Q10 and LCo-Q10 (an anti-inflammatory drug) treatment has shown efficacy in normalizing GFAP through amelioration the astrocyte reactivity and decreasing oxidative stress conditions and may halt the progression of neuro-behavioral abnormalities. A typical neural symptom, abnormal physical exam findings, cognitive testing, and quality-of-life evaluations are also all significantly improved by antioxidant therapy in post-traumatic stress disorder which is consistent with previous study [35].

The genetic causes of autism are largely unknown and no specific biomarker has yet been identified. Previous study has implicated CACNA1C calcium channel mutations in a disorder associated with autism. Genes encoding Ca^{2+} channels as CACNA1C-encoded CaV1.2, an L-type voltage-dependent Ca^{2+} channels (VDCC) are a crucial regulator of dendritic calcium influx in response to synaptic activity and are strongly associated with psychiatric disorders and the pathophysiology. Ca^{2+} signaling channels are essential for a variety of neuronal processes in the CNS, including synaptic plasticity, neurotransmitter release, neuronal excitability, and Ca^{2+}-induced gene regulation [36].

The CACNA1C risk mutation and hippocampus impairment in our study may be caused by higher PPA-induced oxidative stress. Increased CACNA1C expression leads to dysregulated Ca^{2+} signaling and an elevated risk of psychiatric disorders. It stands for an important upstream mechanism that psychiatric disorders use to impair mitochondrial activity.When mitochondrial function is compromised, less ATP is produced than is required for brain activity. It is followed by an increase in CACNA1C mRNA expression and improved calcium signaling, which raise the levels of calcium in the cytosol.

This suggestion is confirmed with other study, which hypothesized that in the affected group, the unregulated Ca^{2+} signaling was responsible for a markedly elevated risk of psychiatric illnesses. Additionally, PPA oxidative stress causes membrane abnormalities in L-type calcium channels that directly affect the channel's shape or the number of channels, which results in an increase in calcium overload and brain malfunction. More evidence for the role of Calcium Channel Genes in ASD stems from the association of ASD with single-nucleotide polymorphism in the *CACNA1C* gene encoding a T-type Ca^{2+} channel subunit [37].

The antioxidant activity in the mitochondria and lipid membranes via scavenging reactive oxygen species (ROS) and their Ca^{2+} buffering activity are the proposed mechanisms through which Co-Q10 and LCo-Q10 are considered to elicit their neuroprotective action. It is thought that an increase in intracellular Ca^{2+} is known to trigger the development of apoptosis.

In particular, at the level of the mitochondria, the decreased

CACNA1C expression produced by Co-Q10 and LCo-Q10 conferred neuroprotective benefits against oxidative stress. Numerous research [38] have looked at the role of Co-Q10 as a neuroprotectant against ROS damage as well as cell death brought on by apoptosis.

In the current study, PPA administration caused histopathological alterations in the hippocampus, cerebral cortex and cerebellum of rats. Remarkably, Co-Q10 and LCo-Q10 protected the hippocampus, cerebral cortex and cerebellum against PPA-induced damage. The LCo-Q10 group had even better results. This is due to the antioxidant neuroprotective effect of both Co-Q10 and LCo-Q10, and increase brain mitochondrial concentrations in animal studies and reduces markers of oxidative damage in the cerebral cortex and hippocampus. The present findings is in agreement with the study reported by [3] that neuroglial activation together with a decrease in the number of cerebellar Purkinje cells and an increase in certain cytokines were investigated in ASD brain tissues. As reported previously, both humans and animals with ASD display altered structures and functions in various brain areas, such as the hippocampus [39] and the cortex [40]. Banker et al. [41] declared that the impaired hippocampal structure and function may affect occurring deficits in social interaction, memory, and spatial reasoning in ASD. Alterations in hippocampal structures; mediate emotion perception and regulation, are related to ASD behaviors [42].

Due to the several essential roles that CoQ10 plays in maintaining healthy cell activity, any imbalance caused by falling levels or full absence upsets the established balance and may have a number of negative effects. CoQ10 has been shown to have antioxidant and anti-inflammatory properties in illness models of Parkinson's disease, acute pancreatitis, and cognitive dysfunction. The biggest problem with CoQ10 supplementation is its low bioavailability upon oral administration, which can be attributed to its high molecular weight, weak chemical stability due to its unsaturated double bond, thermolability, hydrophobicity, and moderate volatility. Significant research has been done to enhance its qualities, including the use of liposomes, which can easily encapsulate hydrophobic medications and increase their bioavailability [43].

The obtained results revealed the neuroprotective role of liposomal loaded Co-Q10 over Co-Q10, via its antioxidant role against PPA-driven oxidation in the brain of autistic rat model.

5. Conclusion

Co-Q10 and LCo-Q10 can be considered a prospective candidate for ASD therapy via regulating GFAP and CACNA1C signaling pathways and oxidative stress pathways. LCo-Q10 therapy was found to be more effective than Co-Q10 in the current investigation. It's possible that liposomes, which are regarded as superior and acceptable drug delivery systems, are to blame for this outcome.

Funding

This study has no financial support.

Ethics

Ethics number in National Research center: 19011.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgment

Authors are grateful for the National Research center for all the facilities and tools provided.

References

[1] M. Elham, G. Mohammad-Ali, A. Mohammad-Reza, S.N. Sahar, K. Wesam, A. Reza, Mitochondrial dysfunction in autistic children and oral coenzyme Q10 supplementation treatment, Autism Open Access 6 (4) (2016), 1000189.
[2] L.V. Natrus, Y.S. Osadchuk, O.O. Lisakovska, D.O. Labudzinskyi, Y.G. Klys, Y. B. Chaikovsky, Effect of Propionic Acid on Diabetes-Induced Impairment of Unfolded Protein Response Signaling and Astrocyte/Microglia Crosstalk in Rat Ventromedial Nucleus of the Hypothalamus, Neural Plast. 22 (2022) (2022), 6404964.
[3] K. Sahin, C. Orhan, S. Karatoprak, M. Tuzcu, P.B.D. Deeh, I.H. Ozercan, N. Sahin, M.Y. Bozoglan, S. Sylla, S.P. Ojalvo, J.R. Komorowski, Therapeutic effects of a novel form of biotin on propionic acid-induced autistic features in rats, Nutrients 17 (6) (2022) 1280, 14.
[4] A.M. Jurga, M. Paleczna, J. Kadluczka, K.Z. Kuter, Beyond the GFAP-astrocyte protein markers in the brain, Biomolecules 14 (9) (2021) 1361, 11.
[5] E. Smedler, L. Louhivuori, R.A. Romanov, D. Masini, I. Dehnisch Ellström, C. Wang, M. Caramia, Z. West, S. Zhang, P. Rebellato, S. Malmersjö, I. Brusini, S. Kanatani, G. Fisone, T. Harkany, P. Uhlén, Disrupted *Cacna1c* gene expression perturbs spontaneous Ca^{2+} activity causing abnormal brain development and increased anxiety, Proc. Natl. Acad. Sci. USA 15 (119) (2022), e2108768119, 7.
[6] W.A. Al-Megrin, D. Soliman, R.B. Kassab, D.M. Metwally, Moneim Ahmed E Abdel, M.F. El-Khadragy, Coenzyme Q10 Activates the Antioxidant Machinery and Inhibits the Inflammatory and Apoptotic Cascades Against Lead Acetate-Induced Renal Injury in Rats, Front Physiol. 7 (11) (2020) 64.
[7] H. Li, F. Chen, Preparation and quality evaluation of coenzyme Q10 long-circulating liposomes, Saudi J. Biol. Sci. 24 (4) (2017) 797–802.
[8] C.L. Kamen, D.L. Zevy, J.M. Ward, I.R. Bishnoi, M. Kavaliers, K.P. Ossenkopp, Systemic treatment with the enteric bacterial fermentation product, propionic acid, reduces acoustic startle response magnitude in rats in a dose-dependent fashion: Contribution to a rodent model of ASD, Neurotox. Res. 35 (2) (2019) 353–359.
[9] H. Attia, S. Albuhayri, S. Alaraidh, A. Alotaibi, H. Yacoub, R. Mohamad, M. Al-Amin, Biotin, coenzyme Q10, and their combination ameliorate aluminium chloride-induced Alzheimer's disease via attenuating neuroinflammation and improving brain insulin signaling, J. Biochem Mol. Toxicol. 8 (2020), e22519.
[10] M. Cleal, B.D. Fontana, D.C. Ranson, S.D. McBride, J.D. Swinny, E.S. Redhead, M. O. Parker, The Free-movement pattern Y-maze: A cross-species measure of working memory and executive function, Behav. Res Methods 53 (2) (2021) 536–557.
[11] N. Sestakova, A. Puzserova, M. Kluknavsky, I. Bernatova, Determination of motor activity and anxiety-related behaviour in rodents: methodological aspects and role of nitric oxide, Interdiscip. Toxicol. 6 (3) (2013) 126–135.
[12] M. Karim, S. Begum, S. Shahzadi, Serum lactate, AST, ALT in male autistic children in Bangladesh, J. Bangladesh Soc. Physiol. 10 (2) (2015) 56–60.
[13] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, AnalBiochem 95 (2) (1979) 351–358.
[14] R. Lefter, A. Ciobica, I. Antioch, D.C. Ababei, L. Hritcu, A.C. Luca, Oxytocin differentiated effects according to the administration route in a prenatal valproic acid-induced rat model of autism, Medicina 56 (6) (2020) 267.
[15] D. Koracevic, G. Koracevic, V. Djordjevic, S. Andrejevic, V. Cosic, Method for the measurement of antioxidant activity in human fluids, J. Clin. Pathol. 54 (5) (2001) 356–361.
[16] M.S. Moron, J.W. Depierre, B. Mannervik, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver, Biochim Biophys. Act. 4 (582) (1979) 67–78, 1.
[17] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method, Methods 25 (4) (2001) 402–408.
[18] Bancroft, J.D. and Gamble, M. eds., (2008). Theory and practice of histological techniques. Elsevier health sciences.
[19] M. Aliashrafi, M. Nasehi, M.R. Zarrindast, M.T. Joghataei, H. Zali, S.D. Siadat, Association of microbiota-derived propionic acid and Alzheimer's disease; bioinformatics analysis, J. Diabetes Metab. Disord. 19 (2) (2020) 783–804.
[20] A. Gvozdjáková, J. Kucharská, D. Ostatníková, K. Babinská, D. Nakládal, F. L. Crane, Ubiquinol improves symptoms in children with autism, Oxid. Med Cell Longev. 2014 (2014), 798957.
[21] R. Mirza, B. Sharma, Selective modulator of peroxisome proliferator-activated receptor-α protects propionic acid induced autism-like phenotypes in rats, Life Sci. 214 (2018) 106–117.
[22] F. Drobnic, M.A. Lizarraga, A. Caballero-García, A. Cordova, Coenzyme Q10 Supplementation and Its Impact on Exercise and Sport Performance in Humans: A Recovery or a Performance-Enhancing Molecule? Nutrients 14 (9) (2022) 1811.
[23] S. Shams, K.A. Foley, M. Kavaliers, D.F. MacFabe, K. Ossenkopp, Systemic treatment with the enteric bacterial metabolic product propionic acid results in reduction of social behavior in juvenile rats: Contribution to a rodent model of autism spectrum disorder, Dev. Psychobiol. 61 (5) (2019) 688–699.
[24] A. Gardner, R.G. Boles, Symptoms of somatization as a rapid screening tool for mitochondrial dysfunction in depression, Biopsy Med 2 (2008) 7.
[25] H.S. Al Salem, H.M. Al Yousef, A.E. Ashour, A.F. Ahmed, M. Amina, I.S. Issa, R. S. Bhat, Antioxidant and hepatorenal protective effects of bee pollen fractions

against propionic acid induced autistic feature in rats, Food Sci. Nutr. 8 (9) (2020) 5114–5127.
[26] A. Alhusaini, L. Fadda, L. Albogami, N. Alnaim, W. Sarawi, D. Mattar, I. Hasan, Liposomal coenzyme Q10 abates inflammation, apoptosis and DNA damage induced by an overdose of paracetamol in rat's liver, J. King Saud. Univ. -Sci. 34 (6) (2022) 102–144.
[27] X. Liu, J. Lin, H. Zhang, N.U. Khan, J. Zhang, X. Tang, L. Shen, Oxidative Stress in Autism Spectrum Disorder (ASD)-Current progress of Mechanisms and Biomarkers, Front. Psychiatry (2022) 162.
[28] F. Erten, Lycopene ameliorates propionic acid-induced autism spectrum disorders by inhibiting inflammation and oxidative stress in rats, J. Food Biochem. 45(10) (2021), e13922.
[29] Z.S. Sangsefidi, F. Yaghoubi, S. Hajiahmadi, M. Hosseinzadeh, The effect of coenzyme Q10 supplementation on oxidative stress: A systematic review and metaanalysis of randomized controlled clinical trials, Food Sci. Nutr. 8 (4) (2020) 1766–1776.
[30] A. Alhusaini, W. Sarawi, D. Mattar, A. Abo-Hamad, R. Almogren, S. Alhumaidan, A. Mahmoud,). Acetyl-L-carnitine and/or liposomal co-enzyme Q10 prevent propionic acid-induced neurotoxicity by modulating oxidative tissue injury, inflammation, and ALDH1A1-RA-RARα signaling in rats, Biomed. Pharmacother. 153 (2022), 113360.
[31] S. Dai, Z. Tian, D. Zhao, Y. Liang, M. Liu, Z. Liu, Y. Yang, Effects of Coenzyme Q10 Supplementation on Biomarkers of Oxidative Stress in Adults: A GRADE-Assessed Systematic Review and Updated Meta-Analysis of Randomized Controlled Trials, Antioxidants 11 (7) (2022) 1360.
[32] Latifa S. Abdelli, Samsam Aseela, A. Saleh, Naser. propionic acid induces gliosis and neuro-inflammation through modulation of PTEN/AKT pathway in autism spectrum disorder, Sci. Rep. 9 (2019) 8824.
[33] S. Jo, O. Yarishkin, Y.J. Hwang, Y.E. Chun, M. Park, D.H. Woo, C.J. Lee, GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease, Nat. Med. 20 (8) (2014) 886–896.
[34] J. Wang, Q. Zou, R. Han, Y. Li, Y. Wang, Serum levels of Glial fibrillary acidic protein in Chinese children with autism spectrum disorders, Int. J. Dev. Neurosci. 57 (2017) 41–45.
[35] J. Jhun, J. Moon, J. Ryu, Y. Shin, S. Lee, K.H. Cho, S.H. Park, Liposome/gold hybrid nanoparticle encoded with CoQ10 (LGNP-CoQ10) suppressed rheumatoid arthritis via STAT3/Th17 targeting, Plos One 15 (11) (2020), e0241080.
[36] S. Lanzetti, V. Di Biase, Small Molecules as Modulators of Voltage-Gated Calcium Channels in Neurological Disorders: State of the Art and Perspectives, Molecules 27 (4) (2022) 1312.
[37] A. Nakao, Y. Matsunaga, K. Hayashida, N. Takahashi, Role of Oxidative Stress and Ca2+ Signaling in Psychiatric Disorders, Front. Cell Dev. Biol. 9 (2021) 222.
[38] C.J. Pastor-Maldonado, J.M. Suárez-Rivero, S. Povea-Cabello, M. Álvarez-Córdoba, I. Villalón-García, M. Munuera-Cabeza, J.A. Sánchez-Alcázar, Coenzyme q10: Novel formulations and medical trends, Int. J. Mol. Sci. 21 (22) (2020) 8432.
[39] Y. Nakagawa, K. Chiba, Involvement of neuroinflammation during brain development in social cognitive deficits in autism spectrum disorder and schizophrenia, J. Pharm. Exp. Ther. 358 (2016) 504–515.
[40] E. Hashemi, J. Ariza, M. Lechpammer, S.C. Noctor, V. Martıńez-Cerdeño, Abnormal white matter tracts resembling pencil fibers involving prefrontal cortex (Brodmann area 47) in autism: a case report, J. Med Case Rep. 10 (2016) 237.
[41] M. S, X. Gu, D. Schiller, J.H. Foss-Feig, Hippocampal contributions to social and cognitive deficits in autism spectrum disorder, Trends Neurosci. 44 (2021) 793–807.
[42] J. Choi, S. Lee, J. Won, Y. Jin, Y. Hong, T.Y. Hur, Y. Hong, Pathophysiological and neurobehavioral characteristics of a propionic acid-mediated autism-like rat model, PloS One 13 (2) (2018) e0192925.
[43] Alhusaini Ahlam, Sarawi Wedad, Mattar Dareen, Abo-Hamad Amjad, Almogren Renad, Alhumaidan Sara, Alsultan Ebtesam, Alsaif Shaikha, Hasan Iman, Hassanein Emad, Mahmoud Ayman, Acetyl-L-carnitine and/or liposomal co-enzyme Q10 prevent propionic acid-induced neurotoxicity by modulating oxidative tissue injury, inflammation, and ALDH1A1-RA-RARα signaling in rats, Biomed. Pharmacother. Volume 153 (2022), 113360.