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Original Research

No Effects of Decanoic Acid on Locomotor Activity and Antioxidant Defences in an Experimental Animal Model of Attention-Deficit/Hyperactivity Disorder

Sonia Carreón-Trujillo[1], Juan Carlos Corona[1,*]

[1]Laboratory of Neurosciences, Hospital Infantil de México Federico Gómez, 06720 Mexico City, Mexico
*Correspondence: jcorona@himfg.edu.mx (Juan Carlos Corona)
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Abstract

Background: Medium-chain triglycerides such as decanoic acid (C10), which is one of the fatty acids that constitute dietary fats, are of substantial interest for their potential therapeutic effects on neuropsychiatric disorders. However, the effects of C10 on attention-deficit/hyperactivity disorder (ADHD) remain to be studied. We explored the effects of C10 on behavioural activity and antioxidant defences in an experimental animal model of ADHD. **Methods**: To establish an experimental animal model of ADHD, neonatal rats were subjected to unilateral striatal lesions using 6-hydroxydopamine (6-OHDA). The rats sequentially underwent open-field and Y-maze tests before treatment [postnatal day 25 (PN25)]. After the subcutaneous administration of either vehicle or C10 solution (250 mg/kg) for 14 days, the behavioural tests were repeated on PN39. Next, we examined the effects of C10 on the expression of the constitutive antioxidant enzymes catalase and glutathione peroxidase-1/2 and the phase II transcription factor nuclear factor erythroid 2-related factor 2 in four different regions of the rat brain. **Results**: Injection of 6-OHDA unilaterally into the striatum resulted in elevated locomotor activity on PN39. The administration of C10 for a period of 14 days did not alter the locomotor hyperactivity. Moreover, the administration of C10 had no significant effects on the expression of proteins related to antioxidant defences in the hippocampus, prefrontal cortex, striatum or cerebellum of both control and lesioned rats. **Conclusions**: The lack of significant effects of C10 in our study may depend on the dose and duration of C10 administration. Further exhaustive studies are needed to verify the efficacy and effects of different doses and treatment durations of C10 and to explore the underlying mechanisms.

Keywords: ADHD; antioxidant defences; behavioural activity; decanoic acid; 6-OHDA

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD), a neurodevelopmental disorder characterised by inattention, impulsivity and hyperactivity, is commonly associated with challenges in academic, social and psychological functioning [1–3]. Although the aetiology and pathophysiology remain unclear, there is increasing evidence suggesting that the disorder is attributable to dysfunctional dopaminergic neurotransmission [4–7]. Additionally, there is evidence suggesting that oxidative stress, neuroinflammation and mitochondrial dysfunction participate in the pathophysiology of ADHD [8–10]. The central nervous system is particularly susceptible to oxidative damage due to its inadequate antioxidant capacity, high energy demand and high polyunsaturated fatty acid content. Oxidative stress can cause tissue and neuronal damage, leading to impaired cellular function and alterations in the properties of cell membranes, which in turn disrupt crucial functions [11]. Antioxidant enzymes can protect against reactive oxygen species (ROS), and the most important constitutive antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [12,13]. Consequently, the extent of oxidative stress is likely to depend in part on both the activity of antioxidant enzymes and the ROS content in a target tissue. Thus, increased oxidative stress has been recognised as a causative factor in the pathogenesis of several neurodegenerative and neuropsychiatric disorders [14,15]. Additionally, the ADHD medication methylphenidate (MPH) has been demonstrated to induce oxidative stress by increasing ROS levels and inflammation and altering antioxidant defences in animal models, and these effects are related to alterations in mitochondrial bioenergetics [16–18]. Furthermore, various studies have reported increases in oxidative stress and alterations of antioxidant defences in children and adolescents with ADHD [19,20]. Moreover, clinical variables such as stress, anxiety and neuroinflammation in ADHD patients are crucial in ADHD pathophysiology [21]. Thus, neuroinflammation and oxidative stress are coexisting factors, causing a worsening in clinical symptoms and triggering a vicious circle.

The ketogenic diet (KD) is a high-fat and low-carbohydrate diet that has been used globally in recent decades as an effective treatment for epilepsy [22,23]. The medium-chain triglyceride (MCT)-based KD is an effective version of the classic KD that permits the consumption of better proportions of carbohydrates and proteins compared with the classical version [24]. MCTs are saturated fatty acids comprising 6–12 carbon atoms. Among MCTs,

caprylic acid (C8) is the most abundant (50%–75% content), followed by the 10-carbon decanoic acid [capric acid (C10), 23%–45% content] and slight amounts of caproic acid (1%–3% content) and lauric acid (1%–5%). Natural dietary sources of MCTs include coconut oil, palm oil and fats [25,26]. MCTs are easily absorbed and metabolised within liver mitochondria through β-oxidation, resulting in the generation of three major ketones, namely, acetoacetic acid, β-hydroxybutyrate and acetone (ketone bodies). MCTs can also cross the blood–brain barrier to reach the brain and serve as alternative energy sources for neurons and astrocytes [27,28]. Additionally, C10 is a ligand for peroxisome proliferator-activated receptor gamma (PPARγ), which is known to be involved in mitochondrial biogenesis and antioxidant defences [29]. The PPARγ antagonist, bisphenol A diglycidyl ether (BADGE) prevents the C10-mediated increase in CAT and citrate synthase levels [30]. Meanwhile, the activation of PPARγ leads to an increase in the expression of SOD, GPx and CAT [31–34]; this activation is also associated with the upregulation of the phase II transcription factor—nuclear factor erythroid 2-related factor 2 (Nrf2) [34–36]. Previously, the MCT diet provoked great interest owing to its indirect or direct effects on epilepsy and energy metabolism [27]. Thenceforth, the MCT diet has been used as a potential treatment for several neurodegenerative and neuropsychiatric disorders; however, the mechanisms underlying its effects remain elusive [27,37]. MCT diet consumption has been reported to reduce anxiety-like related behaviours in rats [38]. Moreover, oral C10 administration in mice resulted in acute anti-convulsant effects at doses that produced plasma exposures similar to those reported using an MCT-based KD in patients with epilepsy [39]. Furthermore, C10 treatment increased SOD, GPx and CAT activities [30,40,41]. However, the contribution and efficacy of the MCT C10 have not been extensively studied. In light of the suggestion that C10 enhances cellular antioxidant defences and alters behavioural activity, this study established an experimental animal model of ADHD-like symptoms. This was achieved by inducing unilateral striatal lesions in neonatal rats using 6-hydroxydopamine (6-OHDA). The primary aim was to investigate the effects of C10 administration on locomotor hyperactivity and detoxifying enzymes within the brains of the rats.

2. Materials and Methods

2.1 Animals and Treatment

Wistar rats (Animal facility, Hospital Infantil de México Federico Gómez, Mexico City, Mexico) were used in all experiments. Pups were housed with their mothers in litters before and after lesioning, and the room was maintained under a constant temperature (22 °C $\pm$ 2 °C) and a 12-h/12-h light/dark cycle. Animals were provided free access to food and water. Rats were handled following the ARRIVE guidelines, and all animal procedures were conducted according to the provisions for animal care and use prescribed in the scientific procedures on living animals and with the approval of the ethical and research committees of our institution (HIM2018-030). Moreover, every attempt was undertaken to minimise the number of animals used and alleviate their distress. Rats were treated with C10 on postnatal days 26–39 (PN26–PN39). The rats were divided into four treatment groups (n = 8 per group): (1) saline-treated control (CN) group; (2) 6-OHDA group; (3) C10 group receiving 250 mg/kg C10 [29] dissolved in Dimethylsulfoxide (DMSO) subcutaneously once daily for 14 consecutive days (PN26–PN39); and (4) 6-OHDA + C10 group receiving C10 on PN26–PN39. Behavioural tests were conducted before and 14 days after treatment, as shown in the schematic diagram of the experimental procedures in Fig. 1A.

2.2 Neonatal Rats Lesioned with 6-OHDA to Establish an Experimental ADHD Model

On PN7, all rat pups were intraperitoneally anesthetised with ketamine–xylazine (50 mg/kg/5 mg/kg) (PiSA Pharmaceutical, Mexico City, Mexico) diluted in 0.9% NaCl. The pups were placed on a stereotaxic frame (Stoelting, Kiel, WI, USA), and unilateral lesioning with 6-OHDA hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was carried out in the right hemisphere. This was accomplished using a 5-µL Hamilton syringe (Hamilton, Reno, NV, USA). 6-OHDA was used at a concentration of 8 µg/µL (calculated from the free base weight) dissolved in a solution of 0.9% NaCl with 0.1% ascorbic acid. The neurotoxin was administered into the dorsolateral striatum at a rate of 0.25 µL/min, with the injection site coordinates adjusted based on a rat atlas for PN7, as follows (relative to bregma, in mm): anterior/posterior, AP = +0.6, medial/lateral, ML = –2.5 and dorsal/ventral, DV = –3.3. Following each injection, the needle was left in place for 5 min to facilitate diffusion and prevent any backflow. Control rats underwent a similar procedure with a saline solution. After lesioning, the pups were kept warm at 37 °C and returned to their mothers until weaning.

2.3 Behavioural Analysis

Prior to conducting each behavioural test, rats underwent a habituation process for the open-field chamber and Y-maze. All behavioural assessments were performed between 17:00 and 19:00 on both PN26 and PN39 for all groups of animals. To ensure a neutral testing environment, the apparatuses were thoroughly cleaned with a 75% ethanol solution before each test to eliminate any lingering odours. Video recordings were made of all behavioural tests and subsequently analysed using Fiji ImageJ software (v.2.0 NIH, Bethesda, MD, USA).

2.4 Y-maze Test

The Y-maze test is widely used to assess working memory. Briefly, the maze was made in a Y-shaped opaque Plexiglas holding consisting of three arms (A–C). The arms

converged in an equilateral triangle (120° from each other) with a width of 15 cm, length of 45 cm and height of 15 cm. Each rat was placed at the centre of the Y-maze and allowed to run freely for 10 min, after which the number of arm entries and the number of alternations were recorded. The alternations were calculated when a rat consecutively entered three different arms, such as ABC and BCA, but not CBC. The percent alternation was calculated using the following formula: number of alternations/(total number of arm entries - 2) $\times$ 100.

2.5 Open-Field Test

The open-field test was performed to assess locomotor activity and was conducted in an apparatus composed of opaque Plexiglas ($90 \times 90 \times 90$ cm^3). Briefly, each rat was placed at the centre of the apparatus and allowed to move freely for 10 min. The total distance travelled in the open-field test was recorded, and the results were indicative of hyperactive properties in the animals.

2.6 Western Blotting

Striatal tissue was obtained from animals in all four groups and divided into ipsilateral and contralateral sides. The cerebellum, prefrontal cortex and hippocampus were homogenised in radioimmunoprecipitation assay (RIPA) lysis buffer (containing an inhibitor cocktail of proteases and phosphatases) for 1 h on ice. The supernatants were collected after centrifugation at 12,000 rpm for 15 min at 4 °C. Protein concentration was determined using Bradford reagent (ThermoFisher Scientific, A55866, Waltham, MA, USA). Subsequently, protein samples were resolved using sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (10%) and transferred to polyvinylidene difluoride (PVDF) membranes (0.22 µM). After blocking with 5% non-fat dried milk for 2 h at room temperature, the membranes were incubated with primary antibodies against catalase, GPx-1/2 and Nrf2 (all 1:500, Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA) at 4 °C overnight. β-actin (1:3000, Abcam, Cambridge, UK) served as a loading control. Later, the membranes were then rinsed thrice in Phosphate Buffered Saline with 0.1% Tween (PBST) and incubated with the corresponding horseradish peroxidase-conjugated secondary antibody (Cat: sc-516102, Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA) for 2 h at room temperature. Bands were visualised via a chemiluminescence signal produced using ECL substrate (Santa Cruz Biotechnology) per the manufacturer's instructions, detected using the Fusion-Solo WL system (Vilber Lourmat, Marne-la-Vallée, France) and quantified using Fiji ImageJ sofware.

2.7 Statistical Analysis

GraphPad Prism Software (Version 8.01, GraphPad, Inc., La Jolla, CA, USA) was used for all statistical analyses. Data are expressed as the mean $\pm$ standard error of the mean of at least eight independent experiments. Differences in protein expression between groups were compared using one-way analysis of variance (ANOVA) with *post hoc* Bonferroni tests. $p < 0.01$ was considered statistically significant for all tests.

3. Results

3.1 C10 Administration did not Alter Locomotor Activity in Rats with Unilateral Striatal 6-OHDA-Induced Lesions

In a previous study, neonatal rats were unilaterally lesioned in the striatum with 6-OHDA, which was employed as an experimental model to investigate ADHD-like symptoms [42] because 6-OHDA–lesioned rats exhibited changes in their behavioural activity. To further investigate this idea, we explored whether rats injected with 6-OHDA exhibit alterations in locomotor activity and assessed the potential benefits of C10 administration. On PN25 (before C10 administration), rats in the 6-OHDA group tended to travel a farther distance than those in the CN group, but the difference was not statistically significant (Fig. 1B). However, rats in the 6-OHDA group travelled a significantly longer distance than those in the CN group until PN39 (Fig. 1B). Nevertheless, the daily administration of 250 mg/kg body weight C10 for 14 consecutive days did not cause a significant reduction in the distance travelled by lesioned rats (6-OHDA+C10 group) versus that travelled by rats in the 6-OHDA group (Fig. 1B). Similarly, C10 administration in CN rats had no effect on the distance travelled (Fig. 1B). Thus, the administration of C10 did not alter locomotor hyperactivity caused by the unilateral injection of 6-OHDA into the striatum.

3.2 6-OHDA and C10 Administration had no Effect on the Spatial Memory of Rats

Because a lack of attention is one of the primary symptoms of ADHD, and as it was previously demonstrated that there is a decrease in spatial attention in this animal model [42], we employed the Y-maze test to investigate the effects of unilateral injection of 6-OHDA into the striatum and the possible ameliorative effects of C10 administration on spatial working memory. No change was observed in the number of arm entries among the groups on either test day (Fig. 1D). Moreover, no significant change was noted in the percent alternation between the 6-OHDA and CN groups on either PN25 or PN39 (Fig. 1D), which is consistent with our previously obtained finding that 6-OHDA injection into the striatum had no significant effects on spatial memory [43]. Additionally, C10 administration for 14 consecutive days did not significantly alter the total arm entries in the lesioned rats (6-OHDA + C10 group) compared with the findings in 6-OHDA rats (Fig. 1C); the percent alternation also did not differ between the two groups (Fig. 1D).

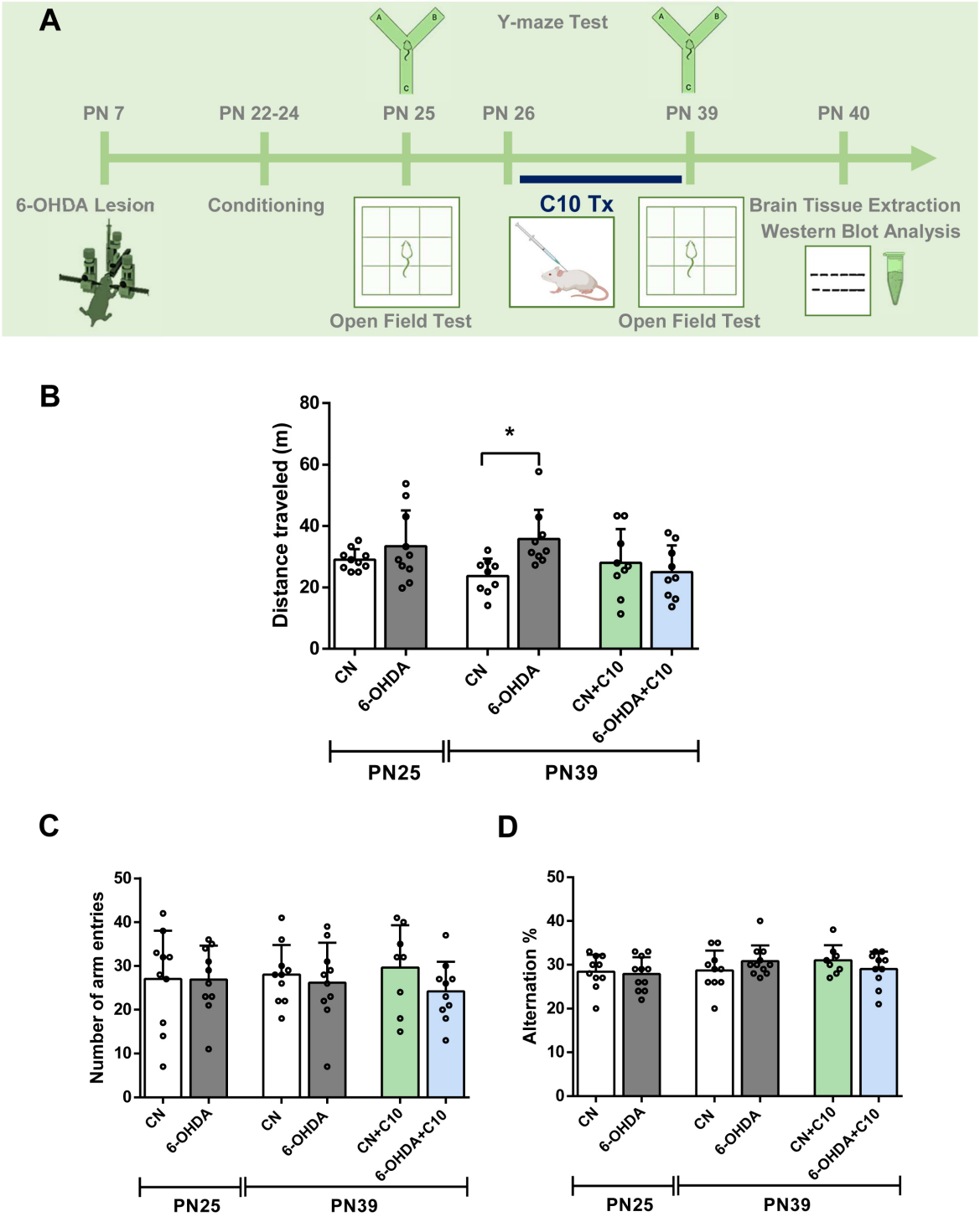

Fig. 1. Unilateral 6-hydroxydopamine (6-OHDA) injection induced locomotor hyperactivity, whereas decanoic acid (C10) administration had no effect. (A) Schematic representation of the experimental procedures and timeline. (B) 6-OHDA–lesioned rats exhibited a significant increase in locomotor activity compared with the findings in control (CN) rats on postnatal day 39, and C10 administration did not induce changes in locomotor hyperactivity. Distance travelled during 10 min in the open-field test. (C) Effects of C10 on the number of arm entries and (D) percent alternation in the Y-maze test. Spatial working memory performance in the Y-maze was not altered by 6-OHDA injection and/or C10 administration. All results are presented as the mean ± standard error of the mean (n = 8 rats/group). *$p < 0.05$ vs. CN group by Student's t-test before treatment and by one-way analysis of variance with *post hoc* Bonferroni tests after treatment. PN, postnatal.

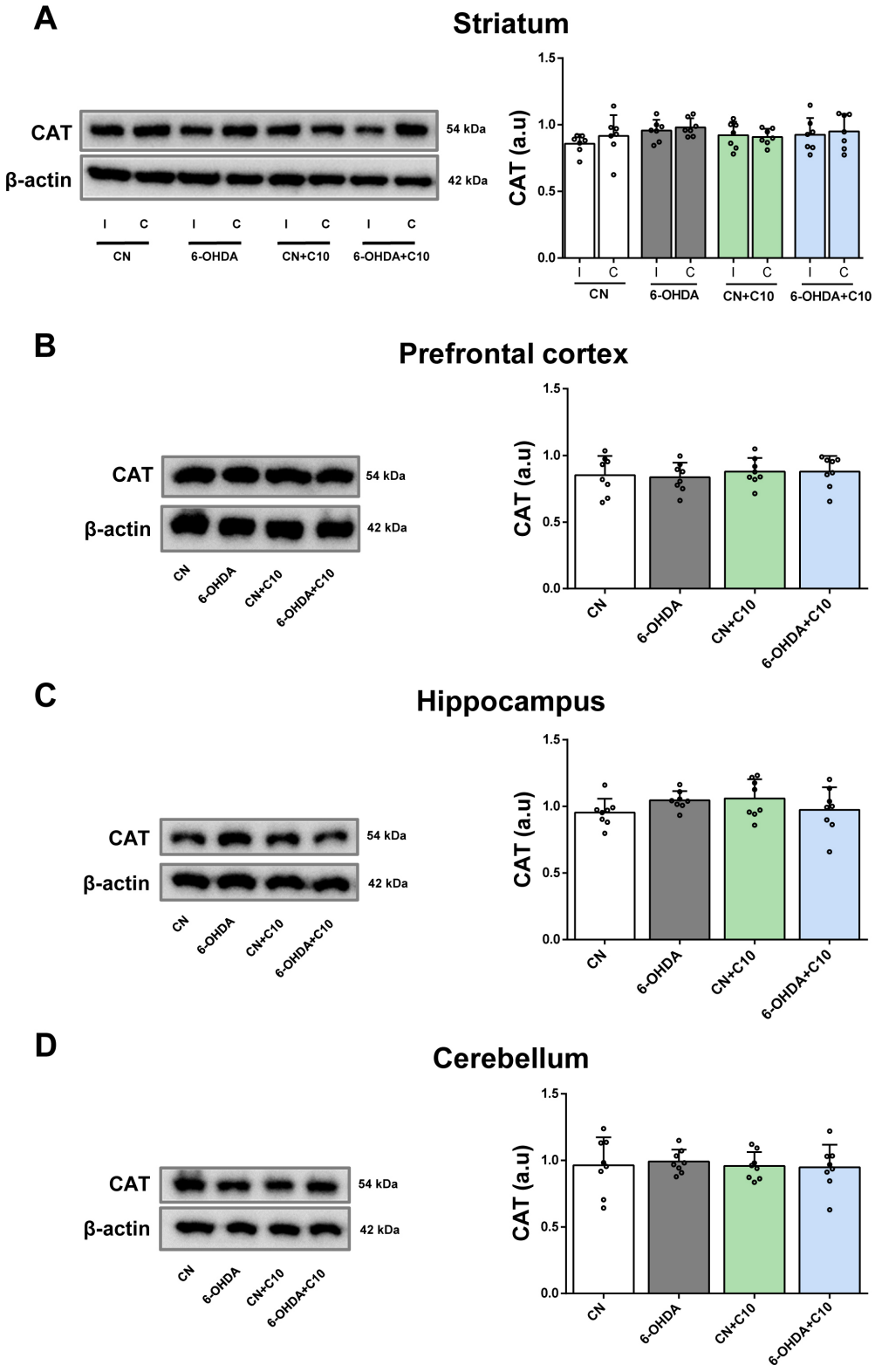

Fig. 2. Chronic effects of decanoic acid (C10) administration on catalase (CAT) expression. Representative western blots and quantification of CAT protein expression in the striatum (A), prefrontal cortex (B), hippocampus (C) and cerebellum (D) in the control (CN), 6-hydroxydopamine (6-OHDA), C10 and 6-OHDA + C10 groups. β-actin was used as the loading control. Results are presented as the mean ± standard error of the mean (n = 8 rats/group). Statistical analysis was performed by one-way analysis of variance (ANOVA). I, Ipsilateral side; C, Contralateral side.

3.3 C10 Administration did not Enhance the Expression of Constitutive Antioxidant Enzymes

To evaluate whether C10 administration altered the expression of the constitutive antioxidant enzymes CAT and GPx-1/2 in the CN and 6-OHDA groups, we measured CAT and GPx-1/2 levels in the hippocampus, prefrontal cortex, striatum and cerebellum via western blotting. We also examined the expression of SOD through western blotting but did not detect specific bands in the different brain regions (data not shown). CAT expression did not differ significantly among the groups in the ipsilateral or contralateral striatum, prefrontal cortex, hippocampus and cerebellum (Fig. 2A–D). Similarly, GPx1/2 expression did not differ among the groups in any of these brain regions (Fig. 3A–D). Conversely, GPx-1/2 in the hippocampus tended to be higher in the 6-OHDA + C10 group compared with the 6-OHDA rats, albeit without significance. Therefore, C10 administration did not enhance the constitutive expression of antioxidant enzymes in the analysed brain regions.

3.4 C10 Administration had no Effect on Nrf2 Expression

Finally, we examined the effects of 6-OHDA and C10 administration on the expression of Nrf2 (a phase II transcription factor) in the prefrontal cortex and cerebellum via western blotting. No significant difference was observed in Nrf2 expression among the groups in either of the brain regions (Fig. 4A,B), indicating that C10 did not enhance phase II detoxification capacity.

4. Discussion

The MCT diet has been widely used for the management of epilepsy [24]. Nevertheless, the effectiveness of the MCT diet and the response biochemical mechanisms remain poorly understood. The potential therapeutic outcomes of MCTs such as C10 on neuropsychiatric disorders or ADHD have not been extensively studied. Therefore, we sought to determine the relative effects of C10 administration on behavioural activity and antioxidant defences in the striatum, prefrontal cortex, cerebellum and hippocampus in an experimental rat model of ADHD.

Experimental rodent models lesioned with 6-OHDA have been used in the last decade to study different aspects of ADHD-like symptoms, focusing on the destruction of dopaminergic projections in the brain [44–47]. This is because deficits in catecholaminergic transmission in the brain represent a pathophysiological cause of ADHD [5,6]. Previously, the neonatal rats lesioned unilaterally into the striatum with 6-OHDA exhibited a reduction in tyrosine hydroxylase (TH) expression in the striatum [42], which is also consistent with our previously reported finding that neonatal rats lesioned unilaterally in the striatum with 6-OHDA exhibited 33% lower expression of TH [43]. The loss of dopaminergic innervation in the ipsilateral striatum of 6-OHDA–lesioned rats resulted in increased locomotor activity (PN39) but no alterations in spatial memory. These data are also consistent with our previous findings [43]. However, several studies analysing the behavioural activity of animals obtained different results because of differences in the sites of 6-OHDA injection, age and evaluation protocols. Previous studies found that lesioning with 6-OHDA in neonatal rodents resulted in increased hyperactivity in young stages, but this hyperactivity disappeared in adulthood (PN36 or PN60) [46,47]. Meanwhile, we observed hyperactivity in our model until PN39. Moreover, C10 administration at a dose of 250 mg/kg for 14 days had no significant effect on locomotor activity, suggesting that the dose or treatment duration of C10 was insufficient to cause a direct effect on behavioural activity. However, there are contradictory results regarding the effects of similar or high doses of C10, KD or MCT diet consumption, either acutely or chronically, on behavioural phenotypes in other experimental animal models. For example, a single oral C10 dose of 1.7–8.6 g/kg had anti-convulsant effects in mice [39]. C10 administered intraperitoneally at a dose of 175 mg/kg in mice 15 min before convulsions delayed the onset of clonic convulsions induced by picrotoxin and increased survival following exposure to a lethal dose of pentylenetetrazole [48]. Diabetic mice that were subcutaneously injected with 250 mg/kg C10 daily for 2–4 weeks exhibited significantly decreased glucose levels and improved insulin sensitivity without inducing weight gain [29]. Feeding treatment (35E% tridecanoin) for 10 days before acute seizure tests exerted anti-convulsant effects in two acute CD1 mouse seizure models [49]. Conversely, 3 months of KD consumption (10% protein and 90% fat) had no effects on locomotor activity, spatial learning and memory, depression-like behaviour and anxiety in naïve adult mice [50]. Moreover, rats consuming an MCT diet (C8 and C10 at a ratio of 40:60) for 15 days did not show improved performance in forced swim tests and social exploration; however, they did exhibit reduced anxiety-like behaviours and improved social competitiveness [38]. Recently, it was demonstrated that oral C10 administration at a dose of 525 mg/kg in adult male mice for at least 21 days did not result in behavioural differences [51]. In the same study, a single oral dose of C10 at a high dose of 5.25 g/kg decreased the distance travelled and increased anxiety-like behaviours in elevated plus maze, open-field and light/dark transition tests. Additionally, a single oral dose of 17.5–175 mg/kg C10 slightly increased the distance travelled in the open-field test. However, none of the results reached study-wide statistical significance [51]. Finally, a prospective open-label study assessed dietary intervention with K.Vita (C10 and C8 at a ratio of 80:20), a medicinal food used for patients with drug-resistant epilepsy, for 12 weeks (median intake of 240 mL in adults and 120 mL in children, representing 19% of the daily energy requirement). While the study was not intended to demonstrate a clinical response, it is worth noting that the mean frequency of seizures or paroxysmal events was significantly reduced, although only measured from the time of their introduction [52].

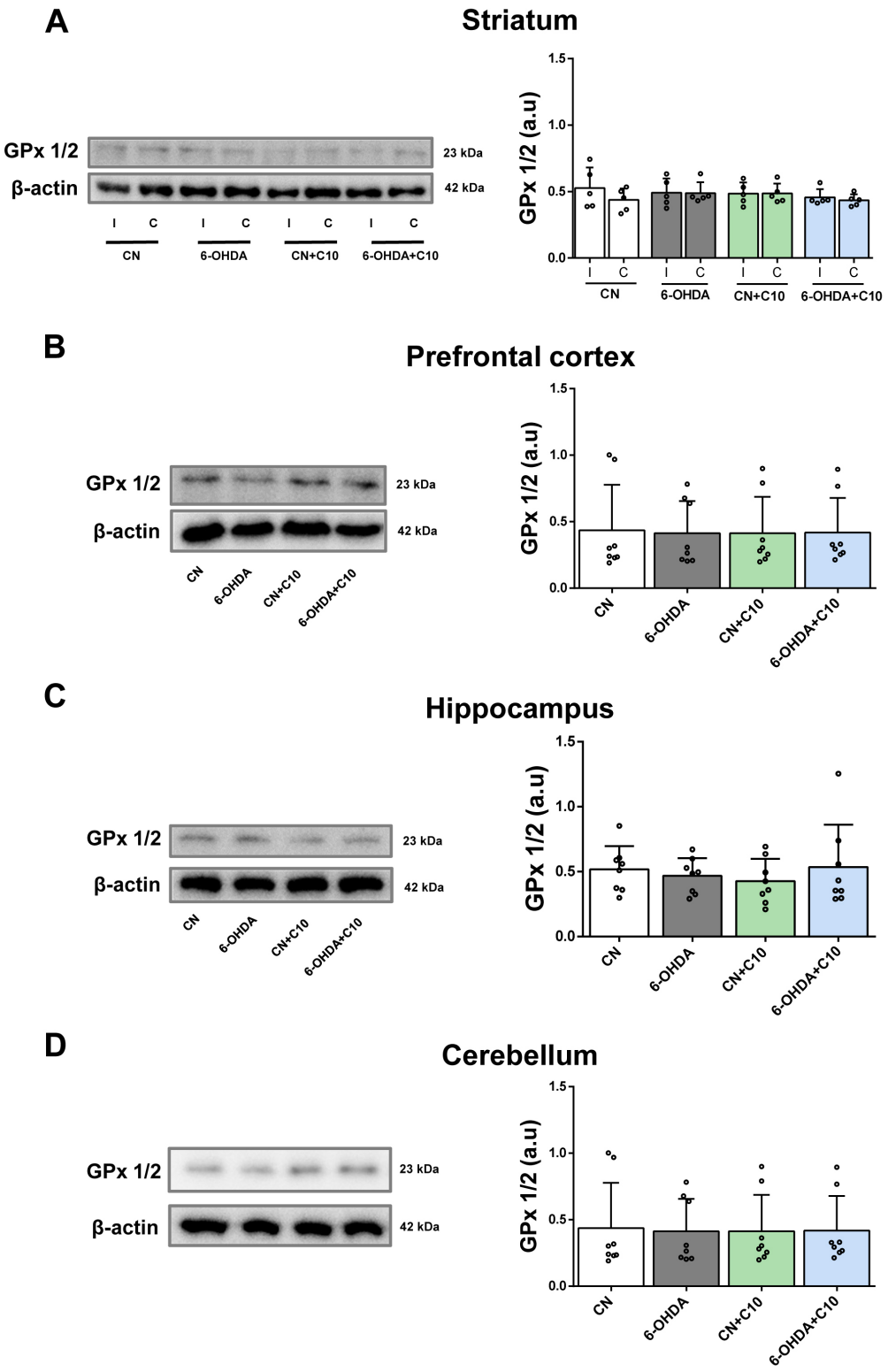

Fig. 3. Chronic effects of decanoic acid (C10) administration on glutathione peroxidase (GPx)-1/2 expression. Representative western blots and quantification of GPx-1/2 protein expression in the striatum (A), prefrontal cortex (B), hippocampus (C) and cerebellum (D) in the control (CN), 6-hydroxydopamine (6-OHDA), C10 and 6-OHDA + C10 groups. β-actin was used as the loading control. Results are presented as the mean ± standard error of the mean (n = 8 rats/group). Statistical analysis was performed by one-way ANOVA.

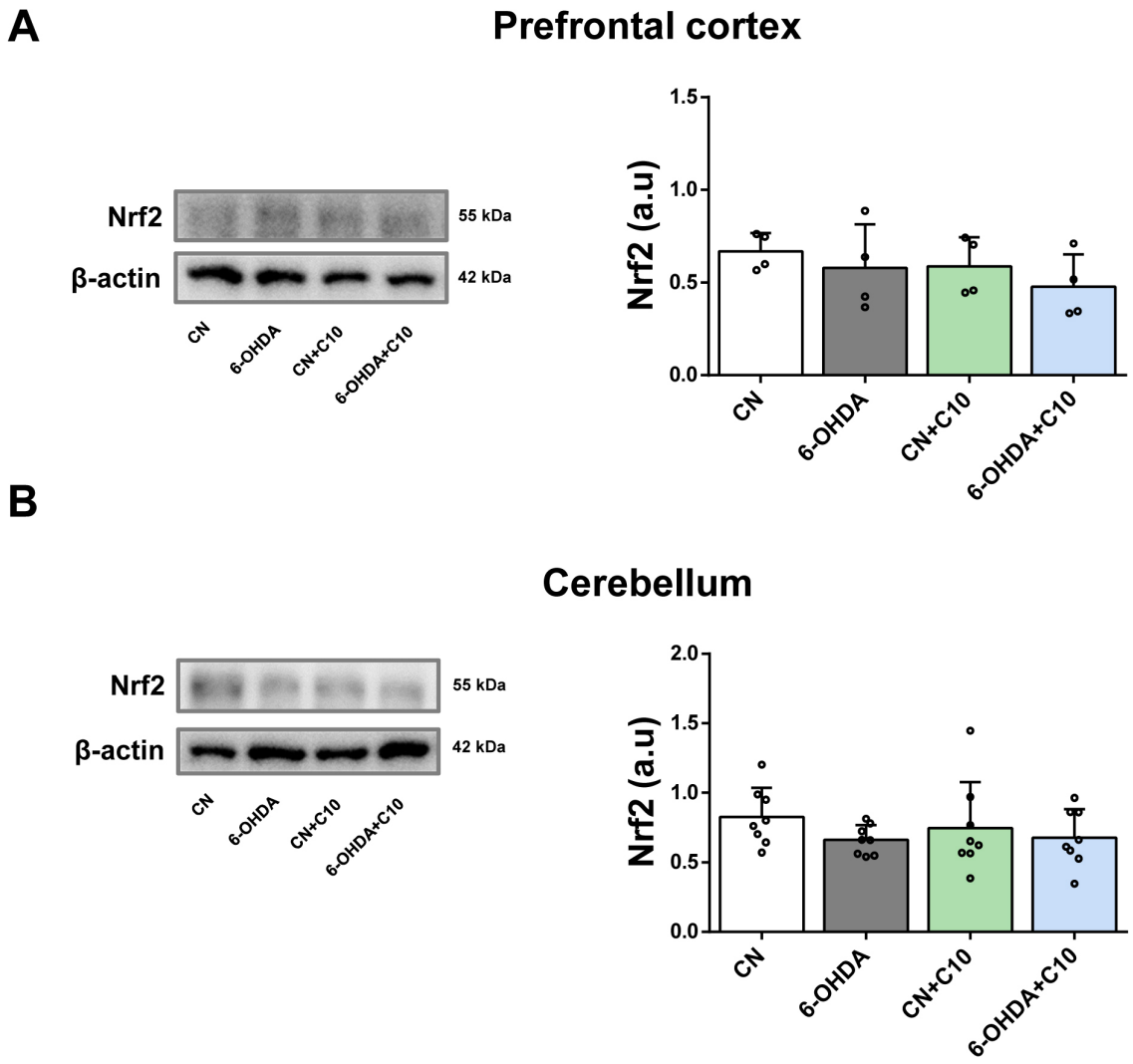

Fig. 4. Chronic effects of decanoic acid (C10) administration on nuclear factor erythroid 2-related factor 2 (Nrf2) expression. Representative western blots and quantification of Nrf2 protein expression in the prefrontal cortex (A) and cerebellum (B) in the control (CN), 6-hydroxydopamine (6-OHDA), C10 and 6-OHDA + C10 groups. β-actin was used as the loading control. Results are presented as the mean $\pm$ standard error of the mean (n = 8 rats/group). Statistical analysis was performed by one-way ANOVA.

Additionally, oral consumption of the KD or MCT diet has been associated with several disadvantages, mainly gastrointestinal disorders, and these symptoms have been reported in several studies. Thus, the use of the KD in people with epilepsy has been associated with vomiting, diarrhoea, body weight loss and constipation [53]. A randomised trial of children with drug-resistant epilepsy treated with the classical KD or MCT diet recorded side effects such as a lack of energy, constipation after 3 months and vomiting after 12 months and found that the MCT diet does not display any advantage over the classical KD in terms of efficacy [54]. In an open-label trial involving patients with epilepsy and the use of K.Vita, the most commonly reported gastrointestinal symptoms included sensations of abdominal bloating or fullness, increased flatulence and constipation; these symptoms were most pronounced during the initial introduction period and gradually diminished over time [52]. Furthermore, mice administered 525 mg/kg C10 orally exhibited a slight but significant decrease in body weights after 2 weeks, and treatment with the highest dose of C10 resulted in marked decreases in body weights after 1 day [51]. These symptoms might be attributable to acidity; thus, C10 should be administered orally suspended in methylcellulose or separately as its respective triglycerides to avoid sodium or acid overload, especially during longer periods of administration. Moreover, alternative administration approaches, such as subcutaneous or intraperitoneal, could be employed in experimental animal models to avoid gastrointestinal side effects.

Oxidative stress, neuroinflammation and alterations in neuronal antioxidant capacity can contribute to the pathophysiology of ADHD [9]. Changes in the activities of antioxidant enzymes such as CAT, GPx, glutathione (GSH) and SOD have been observed in children with ADHD [19,20,55]. The excessive increases in dopamine and noradrenaline levels caused by drugs used to treat ADHD might also induce their autoxidation, increasing oxidative stress and further leading to mitochondrial dysfunction and neuronal damage [9,16,56]. For example, MPH induced oxidative stress and alterations in GPx, GSH and SOD activities, causing neurodegeneration in the brain of rats [17,57].

C10 has been shown to regulate the activity of different antioxidant enzymes, playing an important role in reducing oxidative stress *in vitro*. Thus, C10 treatment at 10 µM for 18 h reduced oxidative stress, thereby attenuating hydrogen peroxide (H_2O_2)-induced cell death in two different neuroblastoma cell lines, and increased CAT activity independent of changes in *CAT* gene expression [40]. C10 treatment (250 µM, a similar concentration reported in the plasma of patients using the MCT diet) for 6 days increased citrate synthase, CAT and complex I activities in SH-SY5Y neuroblastoma cells. In the same study, a PPARγ antagonist prevented C10-mediated increases in citrate synthase and CAT activities in cells [30]. Notably, C10 bound and activated PPARγ in COS-7 cells, and it modulated PPARγ and increased the expression of *PPAR*γ-dependent genes without changing the expression of the *PPAR*γ gene in a diabetic mouse model [29]. The effect of a diet containing C10-enriched mustard oil (200 g/kg during 30 days) was demonstrated by increased activities of SOD, GSH, CAT and GPx in brain and liver tissue and decreased lipid peroxidation in brain or liver tissue or plasma in rats [41]. GPxs are selenoproteins that participate in redox reactions via detoxifying H_2O_2 by reducing it to water [58]. GPx isozymes exhibit different tissue-specific expression and subcellular localisation patterns. For instance, GPx-2 has low or no expression in the human brain. The most highly expressed GPx is GPx-4, followed GPx-1 [59]. In HT22 murine hippocampal cells, treatment with C10 (250 µM for 1 week) resulted in a significant increase of Sirtuin 1 activity, upregulation of mitochondrial respiratory chain complexes and increases in the activities of mitochondrial complexes and citrate synthase [60]. Sirtuins have been shown to regulate key processes such as ROS detoxification, mitochondrial biogenesis and lipid metabolism [61]. Moreover, knockdown of Sirtuin 1 prevented the expression of SOD1, Nrf2 and haem oxygenase-1 (HO-1), the transcription of which is activated by Nrf2, and knockdown abolished the neuroprotective effects of Sirtuin 1 induced by hyperbaric oxygen preconditioning against cerebral ischaemia in rats [62]. In cultured cortical astrocytes, 200 µM C10 increased basal respiration and ATP turnover, whereas in isolated hippocampal mitochondria, tridecanoin treatment increased respiration linked to ATP synthesis and plasma antioxidant capacity in the ferric reducing ability of plasma (FRAP) assay. This treatment additionally increased the hippocampal mRNA expression of *HO-1* and forkhead box protein O1(*FOXO1*) [49]. C10 treatment (200 µM) in mouse brain slices promoted astrocyte glutamine synthesis and stimulated mitochondrial respiration in cortical astrocytes in mouse primary cultures [63]. However, repeated C10 administration for 14 days at 250 mg/kg had no significant effects on the expression of CAT, GPx-1/2 or Nrf2 in any of the brain regions analysed. Considering the acute behavioural effects of C10, these results suggest that the utilised dose of C10 has no long-lasting or cumulative effects on antioxidant defences. Nevertheless, it is vital to assess the impact of C10 on remaining antioxidant activity and its influence on oxidative stress because the standard activity or expression of antioxidant enzymes in brain tissue samples may not accurately represent neuroprotective and intracellular antioxidant capacities. Another possibility is that oxidative stress may be more effectively assessed by measuring parameters other than the levels of antioxidant enzymes. These parameters could include lipid peroxidation in urine or diacron reactive oxygen metabolites (d-ROMs) in serum or plasma, among others. It is also possible that the benefits of C10 administration may have only a marginal impact on oxidative stress. For instance, in most of the aforementioned studies, *in vitro* C10 treatment increased antioxidant activity, but no data regarding related protein expression were reported.

As an energy source and naturally occurring dietary compound, MCTs have demonstrated various properties and functions in metabolism, including the modulation of lipid metabolism, glucose regulation, heat production and hormone secretion [64,65]. Consequently, the cognitive benefits of MCTs as an energy supply for humans have led to their clinical application in treating various neurological and metabolic disorders. They have also been considered for use in healthy individuals as a source of ketone bodies and for their physiological functions [66]. Although the clinical applications of MCTs are promising, there have been inconsistent conclusions in clinical trials, with numerous unresolved questions, such as their impact on the risk of heart disease [65]. The crucial components of MCTs are C8 and C10, and their varying percentages may influence the desired outcomes. Therefore, further studies are needed to understand the underlying mechanisms distinguishing the effects of β-hydroxybutyrate and different MCT types. Given the increasing public awareness and popularity of MCT dietary supplementation for its potential in neurological and metabolic disorders, additional research is necessary to explore how MCTs are used outside of clinical contexts. Moreover, we need to elucidate the long-term effects of early dietary interventions with MCTs on health and disease.

5. Limitations

To serve as a valid experimental model for ADHD, several criteria must be met, including face, construct and predictive validity [67,68]. Consequently, confirming the validity of experimental ADHD models is challenging, and no ideal model exists that can encompass all the core symptoms of ADHD. In recent years, many researchers have developed experimental ADHD models via neurotoxin-induced dopaminergic pathway lesions [47]. Neonatal rodents with dopaminergic pathway lesions induced by 6-OHDA closely replicate numerous fundamental characteristics of ADHD in humans [44–46]. Differences in the dose, volume or injection site of 6-OHDA (whether unilateral into the striatum, intracisternal or into the lateral ventricle) affect the rate of 6-OHDA absorption, which is fundamental for predictive validity. Moreover, the choice of administration route may lead to differences in behavioural outcomes and responses to treatments [42,45,46]. In a previous study, neonate rats with unilateral striatal 6-OHDA-induced lesions were employed as an experimental model to study ADHD-like symptoms [42]. This model demonstrated both face validity (reduction in spatial attention and an increase in locomotor hyperactivity) and construct validity (decrease in TH expression in the striatum) [42,43]. An experimental model can be considered viable even if it does not meet all predictive and face validity criteria. However, it cannot be deemed valid unless construct validity is achieved [68]. Achieving construct validity can be challenging in ADHD due to its complex aetiology and high heterogeneity, which make its neurobiology not fully understood. Despite these challenges, experimental ADHD models, while not perfect reflections of ADHD in humans, can help elucidate the probable underlying mechanisms of ADHD pathogenesis. They can also shed light on the molecular, genetic and cellular mechanisms in various tissues, brain areas, circuits and neurotransmitter systems, which may help identify new therapeutic targets. The limitations of this study include the absence of measurements of dopamine levels associated with 6-OHDA lesion and dopaminergic innervation. However, the previously observed reduction in TH immunoreactivity [42,43] could be regarded as an indirect indicator of dopamine depletion in the striatum. Furthermore, the study identified a decreased count of dopaminergic neurons in the substantia nigra, which resulted from retrograde degeneration induced by the 6-OHDA lesion [69]. This indicates that the 6-OHDA lesion may lead to dopamine depletion significant enough to produce ADHD-like behaviour, contributing to the study of ADHD [47,70]. Additionally, the potential influence of sex differences in the animal subjects was not found to be significant.

6. Conclusions

In conclusion, this study demonstrated that C10 administration for 14 days in 6-OHDA–lesioned rats did not induce changes in locomotor activity, probably because the brain concentration of C10 was insufficient to alter behavioural activity. This study also showed that C10 administration failed to substantially alter the expression of detoxifying enzymes in any of the brain regions analysed. Taken together, these findings suggest that further comprehensive studies are needed to evaluate the expression of antioxidant defences in brain tissue under treatment with C10 at high doses and for longer periods and examine its potential behavioural effects. Although the use of naturally found dietary compounds for various disorders has been considered a healthier and safer approach for patients, they are still far from being considered standard treatments owing to the lack of controlled clinical studies that may well validate both their high efficacy and safety as well their anti-antioxidant or inflammatory properties. Well-designed prospective studies and clinical trials are indispensable for confirming the promise of C10 as an adjunct to standard pharmacological treatments for neuropsychiatric disorders, such as ADHD.

Availability of Data and Materials

The data that support the findings of this study are included in the article; further inquiries can be directed to the corresponding author.

Author Contributions

SC-T performed the experiments and analysed the data. JCC designed the study and wrote the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal study was reviewed and approved by Hospital Infantil de México Federico Gómez, Institutional ethical, Animal Care and Use Committees (HIM2018-030).

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Conflict of Interest

The authors declare no conflict of interest.

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