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Research paper

The protective role of prenatal administration of ascorbic acid on autistic-like behavior in a rat model of autism

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ABSTRACT

Background: Autism is a complicated neurodevelopmental disorder characterized by several behavioral impairments. The pathology of autism is complex and not fully known. Several recent studies have shown alterations in the activities of antioxidant enzymes in autism. Vitamin C is a potent antioxidant that is present in high concentrations in the brain and acts as a neuromodulator. Prefrontal abnormality has been hypothesized to underlie autistic symptoms. The present study investigated the protective effect of prenatally Vitamin C on autistic-like behaviors, oxidative stress status, and histopathological change of prefrontal in valproic acid (VPA) rat model of autism.

Method: The model of autism was induced by subcutaneous administration of Valproic acid (600 mg/kg) to pregnant rats at gestational day 12.5. Vitamin C was administered 600 mg/L in drinking water from the 5th day of gestaion (GD5) up to postnatal day 23 (PND23). Thirty-two rat offspring were divided into four groups: Control, Vitamin C, VPA, and Vitamin C + VPA. The offspring were tested for repetitive behaviors and cognitive ability with a Y-maze task and social interaction with a play behavior task on 31st of Postnatal days. Glutathione (GSH), superoxide dismutase (SOD) activity, and the histological change in the prefrontal lobe were assessed at the end of the study. The number of neurons from the left prefrontal lobe was counted in duplicate from slides stained with hematoxylin-eosin.

Results: In the Y-maze apparatus, spontaneous alteration significantly decreased in the prenatal VPA treated rats compared to control rats showing autistic-like behavior; pre and postnatal Vitamin C treatment increased the alternation indicated benefit effect of Vitamin C. Prenatal VPA treatment impaired play behavior such as sniffing, grooming and darting. Vitamin C treatment attenuated the problems in male offspring social behavior. Histological examination showed an increase in the number of cells in the prefrontal cortex of valproic acid offspring rats compared to other groups. Moreover, prenatal VPA decreased antioxidant enzyme activities in the cortex (PFC) attenuated by Vitamin C administration.

Conclusion: The present study showed that valproic acid induced oxidative stress and neural changes in the prefrontal lobe when administered prenatally which in turn may cause the development of some autistic-like behaviors, and vitamin C may reduce this symptom with its antioxidant effects

1. Introduction

Autism is a complex neurodevelopmental disorder characterized by deficits in executive function, social skills, and sensory processing, as well as restricted interests and repetitive behaviors with different sensory deficits ("American Psychiatric Association: Autism spectrum disorder, 299.00 (F84.0); in: Diagnostic and statistical manual of mental disorders: DSM-5, ed 5. Washington DC, APA, 2013. 2 de la Torre- - Google Search [Internet]. [cited 2021 Jul 6]."). The pathology of autism is complex and not fully known. While autism has a genetic component, environmental risk factors such as chemical toxins and maternal infection during gestation play a role in ASD (Sealey et al., 2016). Several recent studies have shown alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in ASD (Manivasagam et al., 2020). Therefore, oxidative stress probably involved in the pathogenesis of ASD, and the administration of antioxidant molecules may represent a valuable strategy to ameliorate pathological behaviors in ASD patients. The brain has a high metabolic

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rate, making it a readily oxidizable tissue (Erecinska & Silver, 2001). These conditions justify the importance of antioxidants in the brain. Fortunately, a high concentration of Vitamin C is present in CSF and the brain (Harrison, Green, Dawes, & May, 2010). Vitamin C is a potent water-soluble antioxidant with numerous functions, including reactive oxygen scavenging and neuromodulation; it is involved in neurotransmitter synthesis and release in the brain (Figueroa-Mendez & Rivas-Arancibia, 2015). Vitamin C in the brain acts as a co-factor for dopamine beta-hydroxylase in the conversion of dopamine to noradrenaline, and gets involved into the modulation of dopaminergic neurotransmission (Plevin & Galletly, 2020). Numerous authors proposed that dopamine imbalances in specific brain regions could lead to autistic-like behavior (Dichter, Damiano, & Allen, 2012; Paval, 2017). maternal Vitamin C deficiency during pregnancy has shown persistently impaired hippocampal neurogenesis in offspring of guinea Pigs (Tveden-Nyborg et al., 2012).

Vitamin C deficiency in early postnatal life and has shown impaired cognition and reduce the number of hippocampal neurons (Tveden-Nyborg et al., 2009). Another modulatory role of Vitamin C appears to be its histone deacetylation activity (Mustafi et al., 2019). The epigenetic study, showed the enhanced histone acetylation in the prefrontal and temporal cortex in models of ASD (Abrahams & Geschwind, 2010; Sun et al., 2016). Therefore, due to its high concentration in the brain and the histone deacetylation activity, Vitamin C administration during pregnancy can be expected to reduce the incidence of autism. Prenatal multivitamin/folic acid supplement has been reported to reduce the risk of autism spectrum disorders (Braun et al., 2014). A 30-week, Pharmacological dose of ascorbate found a reduction in autism symptom severity as measured by the Ritvo-Freeman scale (Dolske, Spollen, McKay, Lancashire, & Tolbert, 1993).

Exposure to valproic acid (VPA) during pregnancy has increased the risk of autism in children. Like any other disease model, this model cannot be used for the totality of the features seen in human autism ("Frontiers | General developmental health in the VPA-rat model of autism | Behavioral Neuroscience [Internet]. [cited 2022 Jun 26].,"). However, it demonstrates many of the structural and behavioral features observed in individuals with autism. These similarities provide a valuable tool to investigate the etiology of ASD and screen for novel therapeutics (Nicolini & Fahnestock, 2018).

Valproic acid is known as an antiepileptic drug. Its antiepileptic effect is associated with, increased GABA in the brain, and blocked voltage-dependent sodium channels (Romoli et al., 2019). One known pharmacological action of VPA is histone deacetylase (HDAC) inhibition; thus, VPA might increase the risk of ASD through an epigenetic pathway (Mustafi et al., 2019; Tveden-Nyborg et al., 2009). The effect of VPA by the increase of fetal oxidative stress effects mainly the brain rather than other fetal organs. Oxidative stress may be a common mechanism of injury leading to aberrant behavior in the animal model ("Evidence of Oxidative Stress in ASD Derived from Animal Models | Carrie Yochum - Academia.edu [Internet]. [cited 2022 Jun 23]."). Several neuroanatomical abnormalities have been reported to be associated with ASD (Donovan & Basson, 2017). Studies have highlighted that amygdala, hippocampus, cerebellum, and prefrontal regions are structurally distinct in people with ASD (Amaral, Schumann, & Nordahl, 2008).

The involvement of the prefrontal lobe in the neurobiology of ASD has been documented in the literature. The prefrontal lobe has a central role in executive functions and emotion recognition and communication (Phiel et al., 2001); these processes are both compromised in ASD (Courchesne et al., 2011). Brain imaging studies of patients with ASD have shown overgrowth in the prefrontal cortex with an increase in the number of neurons and metabolic activity (Courchesne et al., 2011). Therefore, we tend to determine the potential protective effect of prenatal administration of Vitamin C on autistic-like behaviors by analyzing the alteration of number of neurons and oxidant activity in the prefrontal cortex of valproic acid (VPA) rat model of autism.

2. Material and methods

2.1. Animals

Eighteen pairs of healthy male and female Wistar rats weighing 230–250 gr from the Golestan animal house were selected and placed in separate cages for mating. After mating, a sperm-positive vaginal smear was taken to indicate the first day of pregnancy (GD1). The pregnant rats were separated and divided in three groups of dams. The control group received only normal food and water. B) The autism group received Valproate sodium at a dose of 500 mg/kg on the GD14 (1). C) Vitamin C group that consumed vitamins C (600 mg/L) as drinking water from GD5 of pregnancy up to the 29th day post-partum. approximately three to five male pups were born from each dam. Since Valproate sodium is more likely induced autism in male rats. Only the male pups were selected (Gouda et al., 2022), and divided in to four groups (N = 8). The control group (the first group) was formed by male pups of dams that consumed distilled water as drinking water. The Vitamin C group (the second group) was formed by male pups of dams that consumed vitamins C (600 mg/L) as drinking water from the GD5 up to the 29th day post-partum. The dose of ascorbic acid was chosen according to(Chang et al., 2012). Ascorbic acid was purchased from Osvah Pharmaceutical Co., Iran.

The VPA group (third group) was formed by male offspring of rats prenatally treated with VPA(600 mg/kg subcutaneous) on GD 12.5, followed by receiving normal drinking water ("Frontiers | General developmental health in the VPA-rat model of autism | Behavioral Neuroscience [Internet]. [cited 2022 Jun 26].,"). Finally, the VPA+ Vitamin C group (the fourth group) was formed by offspring of rats prenatally treated with VPA (600 mg/kg subcutaneous), followed by a receiving vitamin C (600 mg/L) in drinking water. Behavioral procedures were taken on PND 30. The experiments were performed between 08:00 a.m. and 2:00 p.m. All the experiments were performed according to the guidelines for laboratory animal use and care set by the Animal Ethics Committee of Damghan University with ethic code (IR.DU.REC.1401.004). the rats keep in a controlled environment (23 ± 2 °C temperature and 45– 65% humidity) and artificially lighted rooms with a 12 h light/dark cycle (lights on at 7:00 a.m., food and water were provided ad libitum). The volume of drinking water consumed by each rat was measured daily This allowed us to ensure that all the rats in each group were getting the same amount of vitamin C.

At the end of behavioral test at PND 32, all male offspring of all groups were euthanized through overdose injection of ketamine/xylazine (n = 8 per group) and brain collected for histology and biochemistry study.

2.2. Play behavior test

Based on the results, the impairment of social interaction is an essential clinical manifestation in ASD (Courchesne et al., 2011). The play behavior test was used to evaluate impaired social interaction. In order to enhance social interactions, the animals were housed separate the night before the experiment. Two animals from the same group, but different litters and cages (VPA vs. VPA, Control vs. Con), were placed into an acrylic plastic circular cage (R, 35 cm) under dim light for 15 min on PND 29. Pairs were tested randomly for groups, and the paired rats did not show a significant difference in body weight of more than 15 g. The following parameters were recorded for 10 min as indicators of social engagement (Tung & Winn, 2011): pinning number (the number of times the resident rat laid on its back and showed his belly to the intruder), dart number (the number of times the resident moved rapidly towards, in parallel, or away from the intruder), the time (sec) spent sniffing the intruder and self-grooming as Stereotype Behaviors.

2.3. Repetitive behavior in Y- maze test

The Y-maze spontaneous alternation test was used to evaluate repetitive/restricted behavior at PND 31 ("Fetal Valproate Syndrome: Clinical and Neuro-developmental Features in Two Sibling Pairs - Christianson - 1994 - Developmental Medicine & Child Neurology - Wiley Online Library [Internet]. [cited 2022 Jan 11].,"). Each rat was placed at the end one arm of Y-maze and allowed to move freely throughout the maze. Five session s were allocated to each rat. For each session, the first choice of the rat (whether the rat first entered the left or right arm) was evaluated. The parameter analyzed was rate of alternation between the right and left arms, which was continuously assessed concerning the arm visited in the previous session. This model is based on rats' natural proclivity, alternating between the visited goal-arms in each trial and a series of successive trials (Dudchenko, 2004). Thus, a higher rate of alternation between the arms was considered normal rat behavior, whereas minor alternation indicated cognitive inflexibility and repetitive behavior. For statistical analysis, the data were transformed into scores: 0 = no alternations, repeatedly visiting the same arm for all five sessions, 1 = one alternation, 2 = two alternations, 3 = three alternations, and 4 = four alternations, constantly alternating between the visited arms for all five sessions.

2.4. Assessment of antioxidant enzymes

At the end of the study, the prefrontal lobe (n = 5 per group) was isolated and prepared as a homogenate using 0.1-M phosphate buffer (pH 7.4). Then, a homogenate was centrifuged at 12,000 rpm at 4◦ C for 20 min. The supernatant was used to determine the oxidative stress markers, including the level of the activity of superoxide dismutase and glutathione peroxidase (GSH-Px), by using methods explained elsewhere. Superoxide dismutase (SOD) activity was measured with a Superoxide Dismutase Assay Kit (Catalog No. KSOD 96, KiaZist Co, IRAN). The activity of SOD was determined by measuring the rate of reduction of cytochrome c at 550 nm (McCord & Fridovich, 1969). Glutathione peroxidase (GPx) activity was measured using the Glutathione Peroxidase Assay kit (Catalog No. KGPX96, KiaZist Co, IRAN). GH-Px activity was determined by measuring the decrease of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm (Dudchenko, 2004).

2.5. Histology: Haematoxylin and Eosin (H&E) staining

The rats were anesthetized with ketamine/xylazine HCl (75/10 mg/kg intraperitoneally) and then were sacrificed on PND 32, and the brains (n = 3 per group) were collected for histological analysis. The brain tissues were immediately collected, and coronal sections of the brain were fixed in 4% paraformaldehyde at 4 °C for 24 h. The brains were processed via dehydration using a graded ethanol series, cleared with xylene, and routinely embedded in paraffin. Six serial coronal sections (5 μm) of three rat forebrain were cut, deparafinized, rehydrated, and then stained with H&E. Finally, the morphology of the prefrontal cortex was observed by light microscope. Images were obtained with a Motic microscope The number of neuronal processes in four microscopic fields (0.107 mm2; 89.82 × 120.70 μm), and diameter (μm) of soma were analyzed by Motic and IT Image Tool (version 3) software.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M (n = 8). The statistical significance level was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests using Graphpad Prism 9 (Graphpad software, San Diego, CA, USA) and the P-value of less than 0.05 was considered statistically significant.

3. Result

3.1. Prenatal VPA treatment induced repetitive/restricted behavior and cognitive inflexibility in male rat offspring

In the Y-maze apparatus, spontaneous alteration significantly decreased in the prenatal VPA-treated rats compared to control rats ($p < 0.001$), suggesting repetitive behavior in the prenatal VPA-treated rats. Vitamin C + VPA-treated rats significantly increased the spontaneous alteration ($p < 0.05$) compared to the prenatal VPA-treated rats, as shown in Fig. 1. Pre and postnatal Vitamin C treatment did not modify spontaneous alternation compared to the control and VPA+Vitamin C group, $p < 0.05$).

3.2. Prenatal VPA treatment impaired play behavior, vitamin C treatment attenuated the problems in male offspring social behavior

Social investigation (sniffing, dart, pining, grooming) is considered an important factor in the social interaction test of rats (Schneider et al., 2008). Prenatal VPA treatment impaired the social behavior of offspring. On the number of pinning (Fig. 2a), Prenatal VPA treatment decreased the pinning number compared to control ($p < 0.001$). Prenatal vitamin C treatment in the VPA group increased the pinning number compared to the group exposed only to VPA ($p < 0.001$). However, the number of pinning remained lower than that of the control group ($p < 0.001$). The group exposed only to Vitamin C did not show a significant difference compared to the control group ($p > 0.05$).

Statistical analysis showed significant differences in the dart number (Fig. 2b). Prenatal VPA treatment decreased the number of darts compared to the control group ($p < 0.001$), and Vitamin C treatment increased the number of darts; however, the number of darts remained

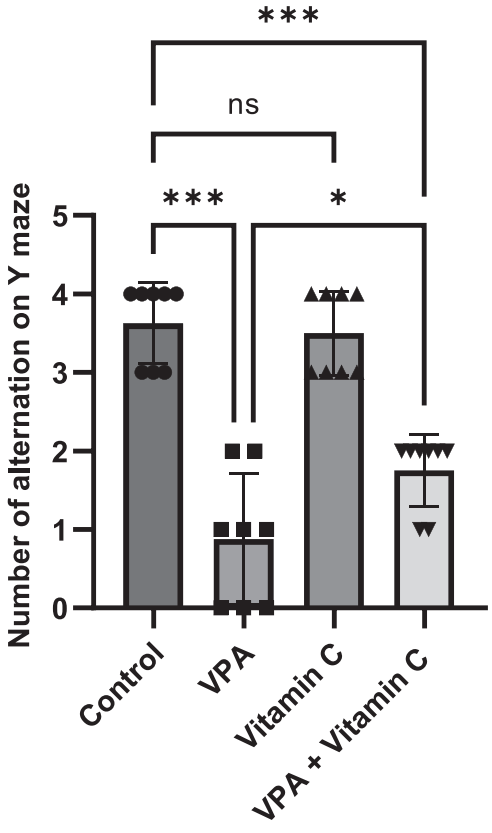

Fig. 1. Effects of prenatal VPA and Vitamin C treatments on Y-maze spontaneous alternation in male rat offspring (n = 8 for all groups). *p < 0.05 * *p < 0.01 * **p < 0.001. The data are shown as the mean ± SEM.

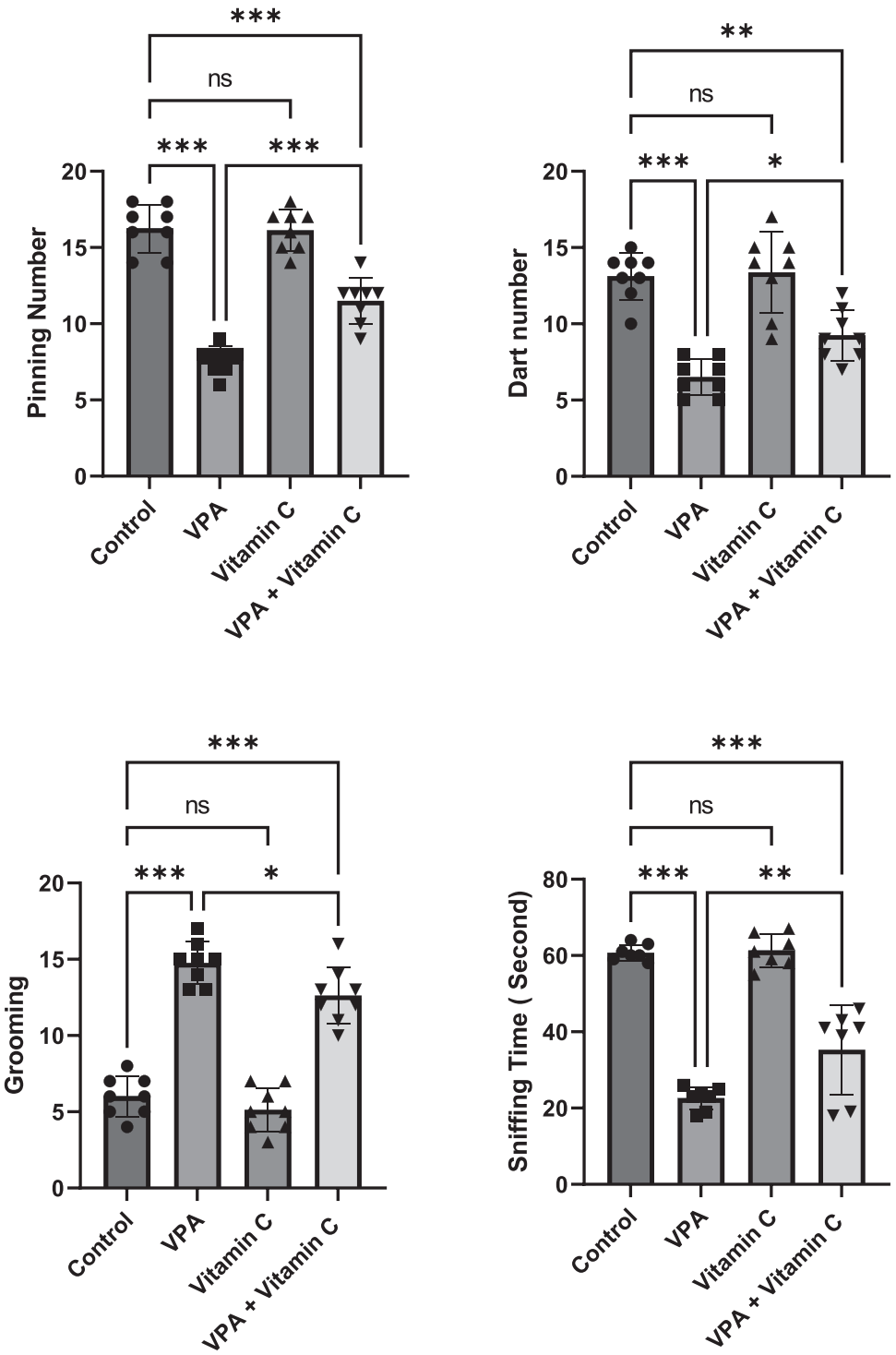

Fig. 2. Effects of prenatal VPA treatment (600 mg/kg) on different parameters measured with the play behavior test in male rat offspring (n = 8 for all groups). *p < 0.05 * *p < 0.01 * **p < 0.001. The data are shown as the mean ± SEM.

lower compared to the control group treatment Fig. 2.b ($p < 0.001$).

Moreover, a one-way ANOVA conducted on the main effect of the prenatal VPA treatment indicated that the VPA-exposed animals significantly increased the number of grooming (Fig. 2c) compared to the control group ($p < 0.001$). The excessive grooming behavior in the VPA-treated group was significantly corrected by Vitamin C ($p < 0.01$); Sniffing (Fig. 2d) decreased both by VPA exposure ($p < 0.001$). However, VPA+ Vitamin C rats had more Sniffing time than the VPA-treated animal's ($p < 0.001$).

3.3. *Effect of VPA on antioxidant activity*

The effect of Vitamin C on antioxidative activity (Enzyme activity in the prefrontal lobe is shown in Table 1. VPA-treated rats exhibit a decreased superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities compared to control group activities ($P < 0.01$). Vitamin C treatment for 40 days significantly increased superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities ($P < 0.05, 0.01$) compared to the VPA-treated rats.

Table 1
Effect of VPA and Vitamin C on the oxidative stress markers, including the activities of superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), (n = 5 for all groups). Data are presented as mean ± SEM. * $p < 0.01$ * ** $p < 0.001$compared to the control and VPA group.

Treatment	Prefrontal Lobe	
	SOD (units/mg protein)	GSH-PX (unit /mg protein)
Control	7.68 ± 0.2	14.23 ± 1.3
VPA	2.45 ± 0.01***	7.82 ± 1.2 * **
VPA + Vitamin C	6.67 ± 0.5	13. 29 ± 2.9
Vitamin C	7.85 ± 0.4	14.91 ± 2

3.4. Prenatal VPA treatment increased number of neurons in PREFRONTAL Lobe

In histological examination (Fig. 3), One-way ANOVA showed an increase in the number of cells in the prefrontal cortex in VPA offspring rats in comparison to other groups ($p < 0.001$). However, their soma size decreased in comparison to the other groups ($p < 0.05$). Moreover, co-administered vitamin C with VPA effectively reduced changes compared to VPA-treated rats ($p = 0.03$) whereas Vitamin C treatment for 40 days did not change the number and size of neurons compared to the Control Group ($p = 0.07$) Table 2.

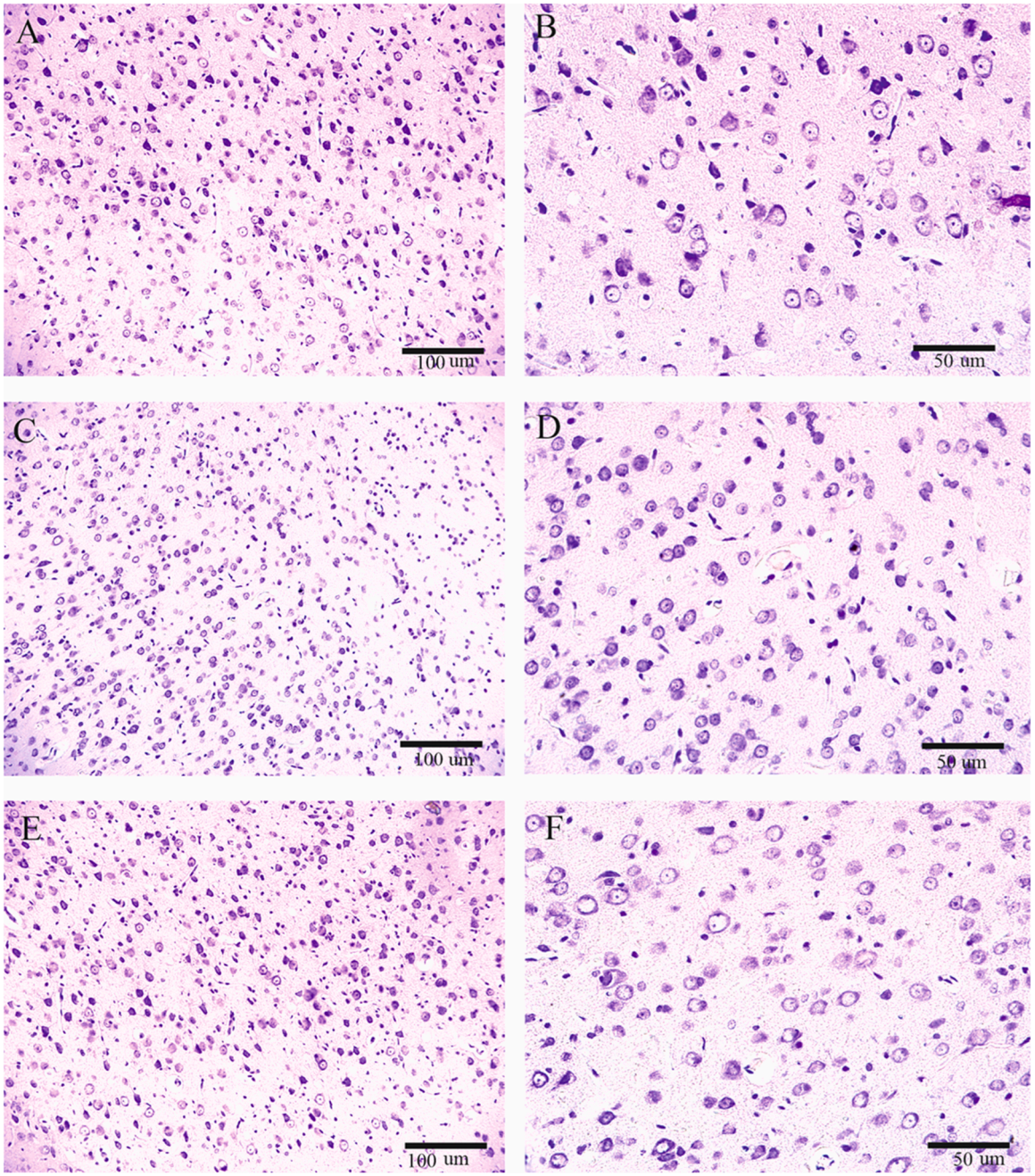

Fig. 3. Photomicrograph showing sections of medial prefrontal cortex stained with hematoxylin-eosin in control group (A, B), valproic acid group (C, D), and vitamin C + VPA group (E, F). (n = 3 for all groups). In the control group, neurons are observed in their normal condition. In the valproic acid group, the number of neurons is more than that of the control group, and their size is smaller. There is no significant change between the vitamin C group and the control group.

Table 2
Total neuronal cell count and soma size in the prefrontal cortex. Data presented as mean + /- SE (n = 3 for each group). * $p < 0.05$ * ** $p < 0.001$ compared to VPA group.

Treatment	Prefrontal Lobe	
	Cell count	Soma size (μm)
Control	80	16 ± 1.32
VPA	120 ± 20***	12 ± 3.2 *
VPA + Vitamin C	88 ± 12	18. 29 ± 2.93
Vitamin C	90 ± 30	15.91 ± 2.06

4. Discussion

The present study aimed to investigate the effects of pre and postnatal treatment of Vitamin C on two important autistics behaviors (i.e., impaired social interaction and repetitive behavior) and assess the antioxidant enzyme activity and the number of neurons in the prefrontal cortex of rats with VPA induced ASD. According to the data, the rat model of autism induced by prenatal VPA was effective and caused impairment in social interaction and repetitive behavior. In addition, prenatal VPA exposure increased oxidative stress due to the reduction of antioxidant enzymes. However, behavioral dysfunction and antioxidants were improved by 40 days of treatment with vitamin c in the rats with VPA-induced autism.

Our studies showed decreases in the social interaction of VPA-treated rats consistent with other results (Kataoka et al., 2013; Schneider et al., 2008). Schneider et al. compared the sociability of VPA-treated rats to unfamiliar animals in an open field area. They reported a decrease in the number of play behavior in VPA rats. The behavioral effects of VPA exposure to rats suggest delays in the development of the early social communications of rats and disturbed sensory system function (Schneider & Przewlocki, 2005). In our result, VPA-treated rats showed increased reentry of the same previously explored arm in a Y-maze, indicating repetitive behavior. Thus, our experimental model of autism is consistent with the literature regarding cognitive inflexibility (Schneider & Przewlocki, 2005). Walcott et al.,(2011) suggested that this effect of prenatal VPA in reducing animal communication is related to changes in neural excitability and synaptic activity (Walcott, Higgins, & Desai). Prenatal exposure to VPA increases the risk of ASD, possibly through its ability to change gene expression via epigenetic remodeling by inhibiting histone deacetylase (HDAC) activity (Phiel et al., 2001). In our study, Vitamin C treatment alleviated restricted behavior and impairments in social play, including pinning, social investigation, and sniffing, in autistic-type rats that resulted from VPA treatment.

An increase in oxidative stress and a decrease in antioxidant capacity in the brain have been implicated as significant contributors to the pathogenesis of ASD (Manivasagam et al., 2020). In our study, we observed a notable reduction in SOD and GSH-Px activity in VPA-treated rats, while pretreatment with vitamin C led to increased SOD and GSH-Px activity in the prefrontal lobe.

The findings by Ibrahim et al. (2016) further support the role of vitamin C in enhancing antioxidant capacity, as they reported an increase in total antioxidant capacity in offspring when pregnant rats were administered oral vitamin C in the presence of formaldehyde exposure. In our research, we hypothesized that the impaired social behavior observed in VPA rats might be attributed to heightened oxidative stress, given the well-established link between valproic acid and increased reactive oxygen species formation and embryonic apoptosis(Tung and Winn, 2011). Studies have previously shown that antioxidant pretreatment or supplementation can protect against embryo toxicity induced by VPA (Zhang et al., 2009).

The brain's oxidative changes can lead to neurochemical disturbances, contributing to neuronal alterations and behavioral dysfunction (Li et al., 2020). Moreover, human studies have demonstrated that certain plasma concentrations of vitamin C are positively correlated with improved cognitive performance and a significantly reduced risk of dementia in older individuals (Hansen et al., 2014).

Considering the potential role of antioxidants in mitigating ASD-like behaviors, it is noteworthy to explore more specific methods to measure GSH-Px activity. As suggested earlier, employing an alternative coupled assay involving glutathione reductase and glutathione substrates could enhance the specificity of GSH-Px activity determination, thereby providing more precise insights into the interplay between oxidative stress and behavioral changes observed in our VPA-treated rat model of ASD.

Moreover, other studies have also suggested a possible role for other antioxidants, such as vitamin C and glutathione, in the prevention or treatment of ASD-like behaviors in animal models(Alinaghi Langari et al., 2021).

One possible explanation for the observed impairments in social interaction and repetitive behavior in rats with VPA-induced ASD is the disruption of the oxytocin system. Oxytocin is a neuropeptide that plays a crucial role in social behavior, including social recognition, social bonding, and maternal behavior. Previous research has shown that prenatal exposure to VPA can lead to a reduction in oxytocin levels, which may contribute to social deficits in individuals with ASD (Modi & Young, 2012).

Vitamin C has been shown to increase oxytocin release in the brain (Chan et al., 2015), which could potentially explain the improvement in social behavior seen in your study. It is possible that Vitamin C's antioxidant properties may have protected oxytocin-producing neurons from oxidative damage, leading to an increase in oxytocin levels and subsequent improvements in social behavior.

The soma size and neuron number of the prefrontal cortex in VPA-induced autism was significantly higher than that of the control group in this study. It is important to note that our imaging was performed at low magnification, which limits accurate measurement of changes in cell body size across different layers. However, these images remain effective in illustrating general trends and variations in cell body size within the prefrontal cortex. The role of the frontal lobe in the neurobiology of ASD has been extensively documented in the literature (Gilbert, Al-Janabi, Tomkins-Netzer, & Lightman, 2017).

Further neuroanatomical evidence of frontal lobe alterations in ASD were reported in a comprehensive review, which described consistent increases of both withe and gray matter in the frontal lobes (Amaral et al., 2008). Brain imaging studies patients with ASD have shown overgrowth in the prefrontal cortex with 67% more neurons in the PFC (Courchesne et al., 2011). Because new cortical neurons are not generated after birth, the increase in neuron numbers in offspring rats with autism points to prenatal processes. Therefore, it is possible that prenatal exposure to VPA induced neurogenesis through its ability to change gene expression by inhibition of histone deacetylase (HDAC) activity. Walcott et al.,(2011) suggested that prenatal VPA's effect in reducing animal communication is related to postnatal changes in synaptic activity and neural excitability (Walcott et al.). Co-administered vitamin C with VPA effectively reduced the number of neurons compared to the VPA-treated rat. The possible underlying mechanism might occur via the decreased oxidative stress, which decreases neuron number increased neuron number in the prefrontal cortex and improves prefrontal-related behavioral functions. In guinea pigs, early life vitamin C deficiency increased oxidative stress and decreased the levels of brain-derived neurotrophic factor (BDNF) in the frontal cortex (FC), resulting in reduced neuron numbers and decreased spine density and dendrite length (Lykkesfeldt, Trueba, Poulsen, & Christen, 2007; "Md, P, Jg S, J L, P TN. Prenatal vitamin C deficiency results in differential levels of oxidative stress during late gestation in guinea pig brains. Redox Biol [Internet]. 2014 Jan 20 [cited 2022 Jul 11];2.,"). Moreover, high levels of BDNF have been reported in the blood of patients with ASD. Vitamin C changes HDAC activity (McCord & Fridovich, 1969; Subramanian, Teafatiller, Moradi, & Marchant, 2021) which is associated with increases in the expression of genes involved in neurogenesis, maturation,

and neurotransmission (Shin, Ahn, Lee, Lee, & Lee, 2004). Lee et al. showed that addition of ascorbate enhanced the differentiation of precursor cells into both neurons and astrocytes over several days in culture (Lee et al., 2003). In another study, 7-day-old newborn mice were injected with ethanol and vitamin C subcutaneously. It was reported that vitamin C could prevent neurodegeneration due to apoptosis in the cerebral cortex (Naseer et al., 2011). In human studies, the specific plasma concentration of vitamin C has been reported to be positively correlated to better cognitive performance and significantly reduced risk of dementia in older people (Hansen et al., 2014).

In conclusion, our results revealed that prenatal VPA exposure caused social deficits and repetitive behaviors, confirming the experimental model of autism. Treatment with Vitamin C can attenuate the characteristic symptoms of autism induced by VPA. The possible underlying mechanism might occur by amplifying the antioxidant capacity which increases neuron number in prefrontal cortex and improved the prefrontal –related behavioral dysfunction. Future studies could investigate the mechanisms underlying these effects, such as changes in gene expression or neurotransmitter signaling, to further elucidate the potential therapeutic benefits of vitamin C for individuals with autism.

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CRediT authorship contribution statement

PM: Study design and concept and drafting. IG: Data collection, literature search, performing the study. HS: Study Design and Concept and Performing the Study.

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