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VITAMIN A DEFICIENCY IMPAIRS COGNITIVE FUNCTIONS IN VALPROIC ACID INDUCED RAT MODEL OF AUTISM

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Abstract

Prenatal exposure to valproic acid (VPA) has been identified to increase the risk of autism in children. However, the multifaceted nature of autism spectrum disorder (ASD) includes other factors, notably vitamin A deficiency. Our study explored whether the conjunction of ASD and vitamin A deficiency intensifies the typical symptoms. We allocated female rats into four distinct groups: VPA alone, VPA combined with vitamin A deficiency (VPA+VAD), only vitamin A deficiency (VAD), and a control group. Specifically, the VPA group was administered a 500 mg/kg VPA injection on the 12.5th gestation day. We assessed various behavioral aspects such as social interaction, memory, and anxiety using specialized tests like the three-chamber social assay, novel object recognition memory (NOR), and elevated plus maze (EPM). Our findings revealed that prenatal exposure to VPA had lasting postnatal behavioral impacts. Offspring from the VPA group exhibited reduced social interactions, heightened anxiety in the EPM test, and displayed memory challenges in the NOR test. Moreover, we observed heightened levels of oxidative stress markers in their brain tissues, and altered enzymatic antioxidant activities. Importantly, the combination of the autism model with vitamin A deficiency demonstrated more severe autism-like behaviors, increased oxidative stress levels, and altered enzyme activities. Conclusively, the overlap of ASD and vitamin A deficiency could amplify ASD symptoms. This suggests that vitamin A deficiency might heighten an individual's vulnerability to ASD. Further investigations are imperative to fully comprehend the interplay between vitamin A deficiency and ASD during neural development.

Keywords: Vitamin A Deficiency, Valproic Acid-Induced Autism, Oxidative Stress, Behavioral Impairment.

1. INTRODUCTION

Autism spectrum disorder (ASD) is characterized by persistent deficits in sociability and communication, as well as restricted and repetitive patterns of behavior and interests [1-3]. In most cases, ASD is diagnosed without any defined etiology. A dearth of knowledge about underlying causes has limited the ability to develop and mobilize effective treatments, and currently only co-morbid manifestations of the disorder can be alleviated (include epilepsy, sleep impairment, sensory abnormalities, and delays and/or deficits in motor functions; psychiatric conditions such as depression, anxiety, irritability and attention deficit hyperactivity disorder [4,5].

Due to the complexity of ASD, numerous animal models have been developed underpinnings the link between metabolic dysfunction and symptoms of autism. Valproic acid (VPA) is a common anti-epileptic drug that displays teratogenic effects. From day 20 to day 24 of pregnancy, before closure of the nerve canal, if pregnant women use teratogenic drugs such as VPA, the probability that their offspring develop a neurodevelopmental disorder increase [6]. The intraperitoneal injection of VPA into female rats during early pregnancy results in autism-like changes in the offspring, and

the brain structures and the biomarker levels in these offspring are similar to those in patients with ASD [7, 8].

However, ASD has been associated with many possible ethological factors (both genetic and environmental). Nutritional factors play an important role in promoting good health, and a preponderance of evidence has linked nutritional deficiencies to an exacerbation of cognitive deterioration. The vitamin A deficiency (VAD) affected all development stages and evoked several malformations [9, 10].

Normally, Vitamin A is involved in neural tissue development and plasticity and its deprivation induced impairment in motor coordination and striatal cholinergic dysfunction [11]. All their receptors for retinoic acid have been shown to be present in the embryonic and adult CNS and exhibit both unique and overlapping patterns of expression in various areas at different developmental stages [12].

However, few studies have identified the links between autism-like and VAD. In view of the potential role of vitamin A in brain development and alter early changes in neural tissue, the present study was conducted in developing Wistar rats pups for checking the effect of the association of VAD and VPA on oxidative markers and behavioral in Autism related behaviors in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Diet

The experimental design of vitamin A deficient diet was the same as described by others [13, 14, 15]. Briefly, the vitamin A-free diet had the following composition (for 100 g of food, dry weight): protein 12.66%, cellulose 5.2%, hydrogenated sunflower oil 4.2%, sucrose plus carbohydrate 68.8%, salt 4.84%, acetic acid 0.2%, vitamin mixture lacking vitamin A 4.1%. The remaining rats continued to be fed the standard laboratory diet containing the same dosage of nutrients and enriched with an additional supplement of vitamin A (15 IU/g), vitamin D (3 IU/g) and vitamin E (10−5 IU/g).

2.2 Animals and treatment

Wistar rats (10 males and 20 females) from the animal house of Ibn Tofail University, Morocco) were kept on a standard diet and water ad libitum after weaning and maintained under a 12-h light/dark cycle at 18–22◦C, relative humidity at 50–60%. 12-week-old female rats (250–300 g), with controlled fertility cycles, were mated overnight. The vaginal secretion was collected in the next morning, and the day in which spermatozoa were detected was designated as the first day of gestation (GD1).

Randomly, dams were assigned into four groups: the VPA, VPA associated with vitamin A deficiency (VPA+VAD), vitamin A deficiency (VAD) or the control group, and the females in VPA group received a single intraperitoneal injection of 500 mg/kg VPA (250 mg/ml in saline, pH 8.3. Sigma, Casablanca, Morocco) while the control ones were injected with saline on GD 12.5, which is the critical period for the end of neural tube closure phase and the beginning of neurogenesis phase [16].

The female rats were housed individually, and were allowed to raise their own litters. The pups were weaned on postnatal day (PND) 21 and separated by sex. Because of the higher male incidence of autism, and impaired social interaction and repetitive behaviors were only observed in all male offsprings after VPA exposure, only male offspring were used in developmental, biochemical and behavioral tests in the current

study from PND 43 to 50.the animals were housed and handled according to The Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, USA 1996, and in accordance with the National Institutes of Health guidelines on the ethical use of laboratory animals for research, using approved protocols by the Animal. The minimal number of animals was used to achieve statistical significance and all efforts were made to minimize their suffering.

2.3 Tissue isolation

Tissue isolation and homogenate preparation Post behavioral testing, the animals were deeply anesthetized with chloral hydrate (100 mg/kg), and their brains were removed from the skull using appropriate bone forceps. Thereafter, hippocampi, cerebellum and prefrontal cortex were dissected out and homogenized in ice-cold lysis buffer (RIPA lysis buffer + 1 mM PMSF) using a Dounce homogenizer. The homogenates were centrifuged at 14,000g for 15 min and stored at 80 C. Total protein levels were later assayed using Bradford reagent.

2.4 Biochemical assay

2.4.1 Determination of Acetylcholinesterase Activity

Acetylcholinesterase (AchE)-specific activity was measured according to the method of Ellman et al [17] using acetylthiocholine iodide (Sigma-Aldrich, USA) as a substrate. The reaction mixture contained phosphate buffer (0.1 M, pH 8.0), acetylthiocholine iodide (0.075 M), and 5,5- dithiobis-2-nitrobenzoic acid (DTNB; 0.01 M) (SigmaAldrich, USA).

After the addition of the brain structure tissue homogenate (prefrontal cortex, hippocampus and cerebellum) (30 min at room temperature), the hydrolysis rate of acetylcholine iodide was measured by a spectrophotometer (Selecta, Spain) at 412 nm. The enzyme activity was expressed as µmol Ach hydrolyzed/min/mg of protein.

2.4.2 Lipid peroxidation assay

Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction in the production of end products such as malondialdehyde (MDA) [18]. Hence, MDA is widely known as a good marker of oxidative stress.

In this study, we analyzed the formation of lipid peroxides by measuring thiobarbituric-acid-reacting substances (TBARS) in cells as previously described by [19]. Briefly, the samples were mixed with 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67%, heated in boiling water for 15 min, and butanol (2:1 v/v) was later added to the solution. After centrifugation (8000 g/5 min), TBARS were determined by measuring the absorbance at 535 nm [20, 18].

2.4.3 Catalase

The activity of the catalase (CAT) is measured at 240 nm using a UV/visible spectrophotometer by the variation of the optical density consecutive to the disproportion of hydrogen peroxide (H2O2). For the enzyme reaction, 20 µl of supernatant was added to 780 of phosphate buffer saline (PBS) (0.1 M, pH 7.4) and 200 µl of H2O2 (0.5 M) [21].

2.5 Behavioral Tests

The behavioral test, including open field, elevated plus maze and three chamber test were conducted in offspring on PND 45. The eye opening, ear unfolding, and for development were inspected for each animal. For the righting reflex (at PND7),the rats were put in their backs and the time taken to turn over to all four paws was recorded. The ears were gently touched with a cotton swab, the score being 0 if there was no response, 1 if there was ear movement and 2 if there was avoidance.

2.5.1 Open-field

The investigatory behavior was tested in a wooden apparatus (100 cm × 100 cm). As previously reported [22], it was enclosed by 40 cm high walls and placed under strong illumination (100 watts, 2 m above the apparatus). The area was divided into 25 squares (20 cm × 20 cm), defined as nine central and sixteen peripheral squares. At the beginning of the 10-min test, the animal was placed in the center of the apparatus and its behavior was videotaped for subsequent analysis. The device was cleaned after each individual rat session. The quantified parameters used in this experiment were the total number of squares visited. [23].

2.5.2 The Elevated plus Maze test

The EPM is an ethological model of anxiety-like behavior in rodents provoked by the novelty and repulsion as a result of elevation and illumination of the maze [24]. This test is based on the creation of a conflict between the exploratory drive of the rat and its innate fear of open and exposed areas; it has been validated for the detection of emotional responses to anxiogenic and anxiolytic substances. Thus, increased open-arm exploration indicates reduced anxiety-like-related behavior. The EPM consists of a wooden plus-shaped platform elevated 70 cm above the floor. Two of the opposing arms (50 cm × 10 cm) are closed by 40 cm-high sides and end walls, having an open roof. In order to avoid falling, the other two arms (open arms) were surrounded by a 0.5 cm high edge.

The four arms had, at their intersection, a central platform (10 cm × 10 cm). A 100-W lamp was placed exactly on the central platform. At the beginning of the test, the rats were placed in the central area of the maze facing an open arm. The behavior was videotaped during 5 min for the time spent in each section of the maze and the number of entries. Time spent in the arms and the number of entries was used to compute time and entries ratio (open/closed) that were used for analysis. Decreased anxiety-like behavior is illustrated by a significant statistical increase of parameters in open arms (time and/or entries).

2.5.3 The sociability test

The sociability test involving in stranger and a familiar rat, performed at PND31, was adapted from [25, 26, 27]. The apparatus is an acrylic plastic box with dimensions (length/width/height in cm) 120/40/50. The box is separated into three chambers, the central chamber being 60 cm in length and each side 30 cm. There is an identical cage in each side chamber, with a stranger rat in one of the cages. The subject rat was allowed free exploration of the different chambers. The test took place in an environment unknown to the rat being tested, in the form of a cage with three communicating compartments.

The right compartment contained an unfamiliar rat from another control litter of the same strain, and the left compartment contained a familiar rat from the same litter as the rat being tested. Unfamiliar and familiar rats were placed in wire cages. At the beginning of the test, the rat being tested was placed in the empty central compartment. The time spent in each compartment and the number of crosses from one compartment to another was measured for 5 min. Time duration and the number of entrances into the chamber with the empty cage, as well as into the chamber with the stranger rat, was analyzed.

2.5.4 Novel Object Recognition (NOR) Task

The apparatus and procedures for NOR training have been described elsewhere [28,29]. The task took place in a 40 × 50 cm2 open field surrounded by 50 cm high walls, made of plywood covered by black fine plastic layer. All animals were given a habituation session where they were left to freely explore the open field for 5 min. No objects were placed in the box during the habituation trial. Twenty-four hours after habituation. NOR training was conducted by placing individual rats for 5 min into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. In a long-term retention, test given 24 h after training. The same rats explored the field for 5 min in the presence of a familiar object (A) and a novel object (B).

A single set of three objects was used for all the animals. All the objects presented similar textures: colors and sizes, but distinctive shapes. The index of recognition memory was defined as the ratio of object B exploration number and the sum of object A and B exploration number. Between trials, the objects were washed with 10% ethanol solution. Exploration of an object was defined as directing the nose to the object at a distance # 1 cm and touching it with the nose; conversely, turning around or sitting on the object was not considered as exploratory behaviour. NOR procedures were conducted in the presence of luminescent source (60 W) from 1 m in the ceiling

2.6 Statistical Analysis

All data are expressed as the means ± SEM. Statistical analysis was performed using Student's t-test to analyze the data between two groups, and one-way ANOVA followed by tukey's post hoc multiple comparison tests using GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA). The criterion for statistical significance was set at $P < 0.05$.

3. RESULTS

3.1. Expression of MDA in different brain areas

VAD-induced autistic like behavior of oxidative damage was assessed by measuring TBARS (indicating MDA levels) in brain cells. The data showed a significant increase in MDA concentration in the prefrontal cortex and hippocampus of VPA-treated male rats as compared to control ($p<0.001$, Fig1. B, C), Compared to VAP–treated groups we showed a significant decrease of MDA levels in cerebellum, prefrontal cortex and hippocampus in Deficient Vitamin A group (VAD) and treated groups with (VPA+VAD) ($p<0.01$ and 0.001, Fig.1 A, B, C). A significant difference in MDA production between vitamin A deficiency and (VPA+VAD) was noted successively in prefrontal cortex and cerebellum ($p<0.001$ and $p<0.01$).

3.2. Catalase Activity

The catalase activity (CAT) was measured in different brain regions in all treated groups. The data showed a significant decrease in cerebellum, and hippocampus (VAD) and (VPA+VAD) treated male rats, and in all brain regions in VPA- treated group as compared to control ($p<0.001$, Fig 2. A, B). Compared to VPA–treated groups we showed a significant increase in this activity in the prefrontal cortex of deficient vitamin A group (VAD) and treated groups with (VPA+VAD) ($p<0.001$, Fig.2 C). A significant difference in (CAT) between vitamin A deficiency and (VPA+VAD) were noted only in cerebellum ($p<0.05$).

3.3. Acetylcholinesterase Activity (AchE)

The Acetylcholine activity (AchE) was measured in different brain regions in all treated groups. The data showed a significant decrease in hippocampus of VPA and VPA+VAD treated male rats compared to control ($p<0.05$ and $p<0.01$, Fig. 3C). Compared to VPA–treated groups we showed a significant increase in this activity in hippocampus of Deficient Vitamin A group (VAD) treated groups ($p<0.001$, Fig. 3C). A significant difference in (AchE) between vitamin A deficiency and (VPA+VAD) were noted only in the same brain area ($p<0.01$, Fig 3C).

3.4. Postnatal developmental and behavioral tests

3.4. 1. Turn over time and eye opening

The Acetylcholine activity (AchE) was measured in different brain regions in all treated groups. The data showed a significant decrease in hippocampus of VPA and VPA+VAD

3.4. 1. Gestational VAD aggravated autism-like behaviors in the VPA-induced rat model of autism

Autism- related behaviors were tested in all rats, as shown in figure (5). The VPA exposure affect significantly the time spent in central area -of open field test- compared to controls ($p<0.001$), the same data was observed when the Vitamin A deficiency was associated to VPA ($p<0.001$) or alone ($p<0.01$) (Fig. 5A). Conversely, the number of crosses in VPA groups was reduced, when compared to controls; however, this decrease was not significant even between all studied groups (Fig 5B).

As shown in figure (5C), the prenatal VPA exposure induced anxiety–like behavior evidenced in significant decrease in time spent in open arms of elevated plus maze (EPM) ($p<0.001$), the following observation was also shown in vitamin A deficiency associated to VPA (VPA+VAD) ($p<0.01$) or vitamin A deficiency alone(VAD) compared to controls ($p<0.001$). No significant change was observed when compared VPA groups to (VPA+VAD) and (VAD) groups.

In the three chamber test, the VPA group spent significantly more time in the familiar rat chamber and less time in the stranger rat chamber than the control group ($p<0.001$). Same data was observed in the V and VPA+VAD treated male rats regarding the time spent in the Stanger rat chamber compared to control ($p<0.01$). However, no significant differences were observed between VPA, VAD and VPA+VAD groups (Fig. 5D). During the retention session, all the rats were able to differentiate between the two objects (the familiar and the new). However, there is no significant difference in the time of exploration between the new and familiar in the same treated groups except for controls. Indeed, the exploration time of new object was significantly

decreased in VPA groups compared to controls (p>0.05). However, this exploration time was increased in VAD and VPA+VAD, but remains without apparent significance compared to VPA and control groups (Fig. 6E). Similarly, the object recognition index (RI) seems to be altered in all the treated groups. Certainly, the (RI) is above the threshold of recognition (more 50%) in the control group, but this index was reduced in VPA (7%, p<0.05), VAD (13.5%, p<0.01) and VPA+VAD (18%, p<0.01) groups compared to controls (Fig. 5F).

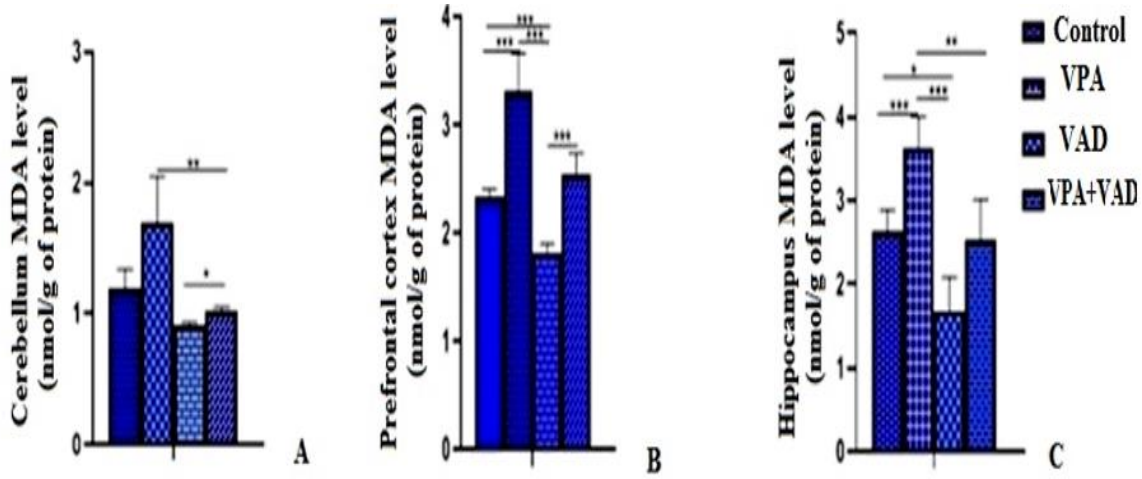

Figure 1: Oxidative damage in brain regions of valproic and vitamin A deficiency induced autistic like behaviors in male wistar rats. The cerebellum (A), the prefrontal cortex (B) and hippocampus (C). (Control, n=8), valproic acid (VPA, n=8), Vitamin A deficiency (VAD, n=8), and (VPA+VAD, n=8) groups. Results are represented as mean ± SEM and expressed in nmol/g of protein. The significance level is 0.05. ** p< 0.01, *** p < 0.001

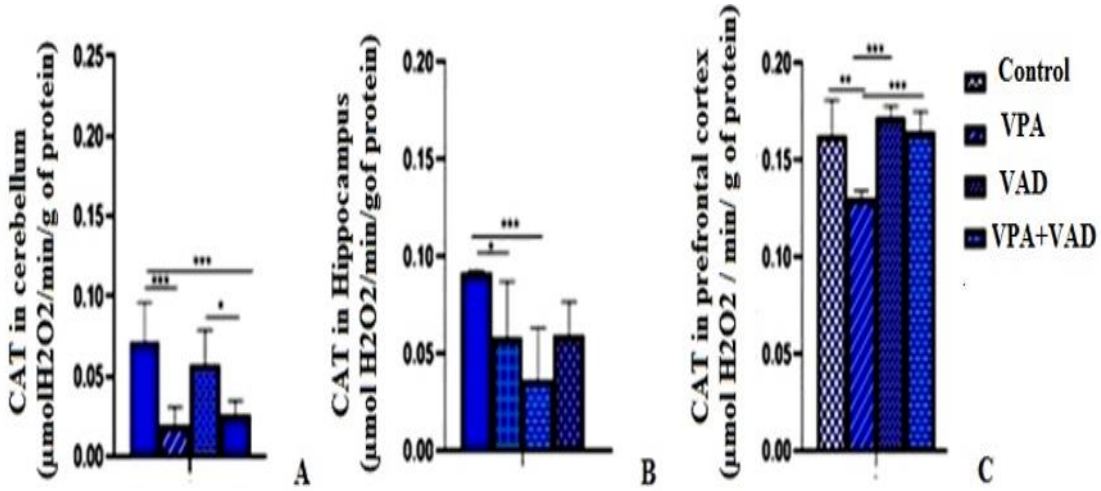

Figure 2: Effect of valproic and vitamin A deficiency induced autistic like behaviors in male wistar rats on catalase activity in different brain areas. The cerebellum (A), hippocampus (B), the prefrontal cortex (C) and (Control, n=8), valproic acid (VPA, n=8), Vitamin A deficiency (VAD, n=8), and (VPA+VAD, n=8) groups. Results are represented as mean ± SEM and expressed in µmo H2O2/min/g of protein. The significance level is 0.05. ** p< 0.01, *** p < 0.001

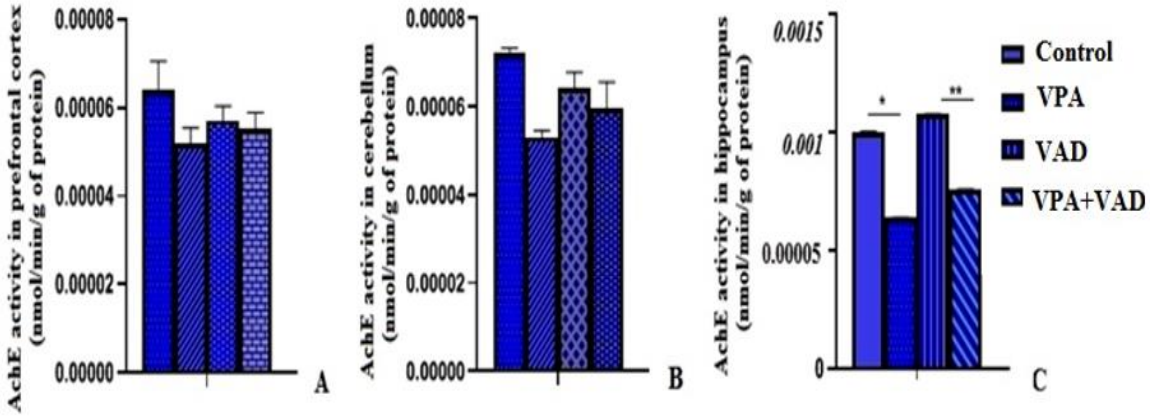

Figure 3: Effect of valproic and vitamin A deficiency induced autistic like behaviors in male Wistar rats on acetylcholinesterase activity in different brain areas. The cerebellum (A), hippocampus (B), the prefrontal cortex (C). (Control, n=8), valproic acid (VPA, n=8), Vitamin A deficiency (VAD, n=8), and (VPA+VAD, n=8) groups. Results are represented as mean ± SEM and expressed in µmol H2O2/min/g of protein. The significance level is 0.05. ** p< 0.01, *** p < 0.001

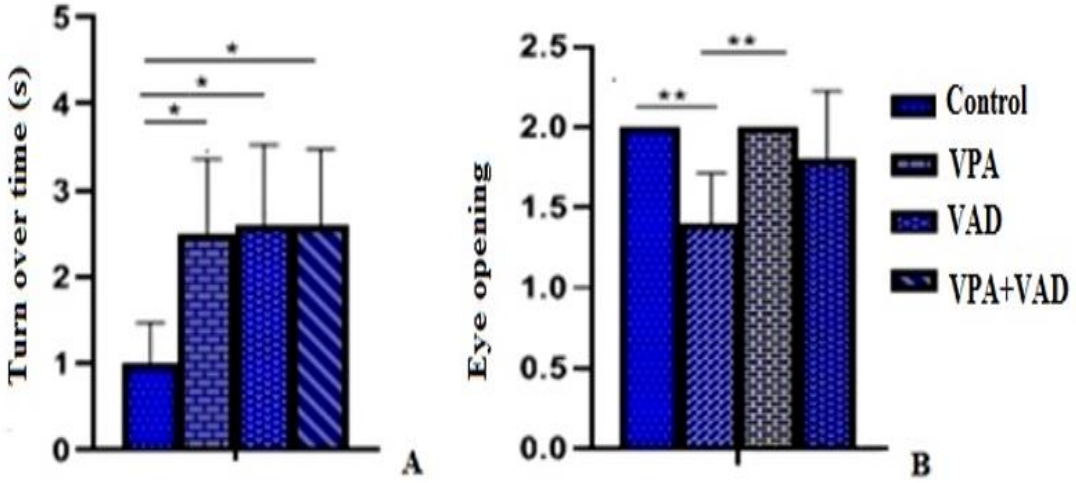

Figure 4: Effect of valproic and vitamin A deficiency induced autistic like behaviors in male wistar rats on postnatal maturation development. (A) Turn over time in righting reflex, (B) eye opening. Results are represented as mean ± SEM, the significance level is 0.05. * p< 0.05, ** p<0.01

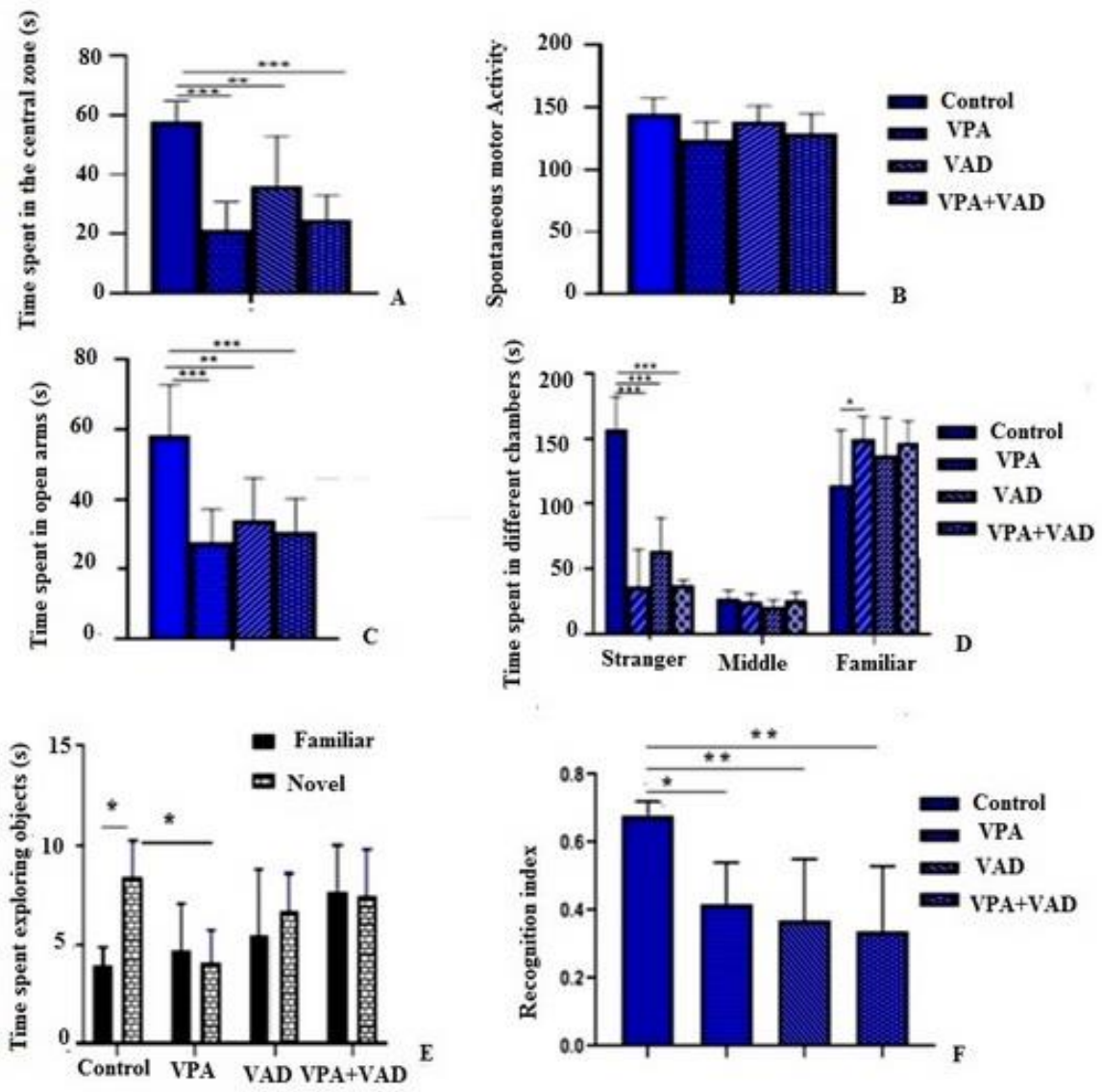

Figure 5: Autism related behaviors in valproic acid (VPA, n=10), Vitamin A deficiency (V, n=10) and (VPA+V, n=10) groups, controls (n=10). (A) The time spent in the central zone and, (B) spontaneous motor activity in the open field test. (C) Time spent in the open arm in elevated plus maze. (D) Social preference between familiar rats and strangers in the three chambered apparatus. (E) Object scanning time for long term memory, (D) The object recognition index for long-term memory in object recognition apparatus. Results are represented as mean ± SEM, the significance level is 0.05. * p< 0.05, ** p<0.01, *** p < 0.001

4. DISCUSSION

The results of the behavioral experiments demonstrated that prenatal exposure to VPA (500mg/kg) at embryonic day 12.5 had long-term effects on postnatal behaviors in rats. VPA exposed offspring neither exhibited a significant reduction in social interaction, nor displayed enhanced anxiety-related behavior in the EPM test and exhibited poor memory object index in NOR task. All behavioral changes observed in the present work are in line with previous studies reporting the induction of ASD-like

behaviors in the off springs following prenatally VPA treatment [30, 31]. In addition to behavioral alterations associated with VPA exposure in utero, there were also changes in physical phenotype such as crooked tail. Recently, it has been reported that embryonic exposure to VPA causes developmental malformations including tail deformity (i.e. twisted and/or bent tail) more frequently in males, which may be associated with neural damage [32,33]. The eye-opening pattern described in our results also suggests maturational delay in prenatal exposed VPA rats. This delayed eye opening time also support impaired glutamatergic synapse maturation in the superior colliculus [34,35,36]. Summarizing our findings of these developmental and behavioral parameters of our study, the autistic pups (VPA model) showed delayed attainment of developmental milestones when compared to the control group in terms of righting reflex, eye opening and tail deformity. Delayed attainment of development milestones and social interaction deficits showed in our results well indicated that the animal model of autism was successfully replicated.

The VPA-treated rat showed an increased level of oxidative stress. We found an increased level of MDA in various brain tissues including prefrontal cortex, hippocampus. Lipid peroxidation was also higher in the VPA-treated offspring. Moreover, enzymatic antioxidants such as CAT were depleted with the exposure of VPA at prenatal stage. Similarly, CAT level was reduced in the brain structures including prefrontal cortex, hippocampus and cerebellum of VPA-treated group. These high levels of MDA suggest the existence of an elevated damage to the lipid component of the cell. The MDA concentration correlates directly with ROS – mediated cell damage, specifically OH [37]. This damage may reduce the viability of the hippocampal cells [38] associated with learning, memory, and neuronal communication of the prefrontal cortex which is important in social interaction and cognition. Findings from the present study showed that the prenatal administration of VPA increased MDA as well as a decrease in antioxidant enzyme activities in the prefrontal cortex and hippocampus. These observations clearly illustrated an elevated level of oxidative stress that interferes with the early stages of brain development in the prenatal VPA-exposed rats. On the other hand, there are few studies that have examined the diagnostic value of oxidants and antioxidants in psychiatric disorders. Camkurt et al. [39] Reported that increased plasma MDA levels were very valuable markers for diagnosing major depression [40], schizophrenia [41]. However, it is still uncertain whether the changes seen in peripheral MDA and CAT levels will be repeated in the central nervous system. However, it is shown that neural cells are more sensitive to the effect of oxidative stress during the early stages of brain development. Our findings are consistent with the recent reports [42, 43, 44] regarding neurodevelopment disorders in autism and increased lipid peroxidation in brain structures notably cerebellum and cortex in autistic children [45,46].

The early impairment of social functioning may reflect the existence of the cholinergic insult in the early development of ASD. Our results indicated that exposure to VPA at prenatal stages decreased the activity of AChE possibly involved in the degradation of ACh at muscarinic and nicotinic synapses. In addition, decreased concentrations of choline, a precursor of acetylcholine synthesis and nicotinic-cholinergic receptor (nAChR) agonist have been reported in autistic patients [47, 48]. In this study, in utero exposure of rats to VPA increased acetylcholinesterase activity in the hippocampus and cerebellum via hydrolysis of acetylcholine indicative of cholinergic deficit. This reduction in AChE activity could be related to abnormal locomotor activity measured

in the treated animals. Our behavioral tests in the open field did not show abnormal locomotor activity, whereas AChE activity was significantly inhibited in the cerebellum, the brain structure involved in reflex adjustments and motor control [49].

In our study, we developed a rat model with gestational VAD based on the VPA induced autism. Vitamin A was found to be the most seriously deficient nutrient in children with ASD in earlier study, it shows that a low serum VA level may be a risk factor for ASD [50,51]. It seems that Vitamin A exerts its effect through its main derivative, retinoic acid (RA), by inducing or repressing transcription of genes through binding to specific nuclear receptors of the steroid/thyroid hormone superfamily of transcriptional activators. Three isotypes of the RAR family, α, β and γ, which have shown to be present in the embryonic and adult CNS and exhibit both unique and overlapping patterns of expression in various areas and at different developmental stages. Following vitamin A supplementation, RARα and γ mRNA expression levels were significantly enhanced, and the expression level of RARβ exhibited an increasing trend [51]. From this evidence the author speculated that the improved symptoms of autistic children may be due to the modulation of transcriptional activity of certain downstream genes by RAR-mediated signal transduction.

Based on the reporting data, in the brain, the VAD affected all development stages and evoked several malformations [9,10]. Vitamin A deprivation induced severe locomotor deficits and impaired motor coordination and striatal cholinergic dysfunction [11] impaired cholinergic transmission [52] and damaged structure of CA1 neurons, impaired somatostatinergic system [53-55]. Our study revealed that autism model associated to VAD at gestational stage exhibited more severe autism like behavior especially social interaction deficits, increased level of oxidative stress and AChE activities. These results are in line with others, Guo et al. (2018) also demonstrated that vitamin A deficiency exacerbates ASD symptoms in patients because it plays an important role in numerous biological pathways such as differentiation, proliferation, and development of the vertebrate central nervous system. It has been reported that VAD is capable of increasing the level of oxytocin via the CD38 process pathway in ASD patients [56,57], and therefore, brain activity and social abilities significantly may be increased through the oxytocin in the autism patients [58,59]. Also, it revealed that supplementation with vitamin A could be an acceptable therapy for ASD patients to help in the maintenance of various cellular biochemical reactions in children with autism. On other hand, more studies had shown that Vitamin A emerged as an oxidative stress marker in induced toxicity studies [56,60].

5. CONCLUSION

In conclusion, the association of ASD and nutrient deficiency notably vitamin A could exacerbate the ASD pathogenesis. The evidence indicates that oxidative stress is an integral part of the pathophysiology of ASD and is linked to the severity of the characteristic symptoms exhibited by children having ASD. Taken into account the potential role of vitamin A and oxidative status of children with ASD, we suggest that these two parameters can be used as biomarkers to elucidate the mechanism by which VAD and ROS induce ASD during neurodevelopment. Moreover, many further research studies are needed to understand how to improve the child's symptoms and alleviating their suffering.

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