Received: 8 December 2023 | Accepted: 22 June 2024 Nig J Neurosci 15(2):48-61.2024

Nigerian Journal of Neuroscience

https://njn.nsn.org.ng

DOI: 10.47081/njn2024.15.2/002

Original Article Open Access

Evaluation of the Therapeutic Potential of Prenatal Morin Supplementation on Valproic Acid-Induced Autism in Offspring of Epileptic Pregnant Wistar Rats

Chike I. P. Anibeze[1], Vivian O. Ojiakor[1]*, Ignatius I. Ozor[2]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Parklane, Enugu, Nigeria
[2]Department of Surgery, College of Medicine, Parklane, Enugu, Nigeria

ABSTRACT

Anti-epileptic drugs such as valproic acid (VPA) have been reported to have detrimental effects on the offspring of women with epilepsy (WWE). This study investigated the effects of prenatal morin supplementation on the hippocampus of a rat model of autism, prenatally induced by VPA. The pups of adult female kindled and non-kindled rats were used for this study. Pregnant rats were randomly assigned to five groups (n=6): Non-kindled rats' pups were assigned as the control group and received normal saline (mL/kg). The kindled rats were allowed to mate fourteen days after kindling and were separated into groups as follows: pentylenetetrazol (PTZ), PTZ+VPA, PTZ+VPA+morin, and PTZ+VPA+folic acid. Treatments were carried out on gestational days 8–18. Following delivery and weaning on postnatal day 32, neurobehavioural studies were conducted. Results showed that *in-utero* morin supplementation improved all VPA-induced behavioural and cognitive deficits in the offspring of kindled Wistar rats. Furthermore, prenatal VPA increased malondialdehyde and nitrite levels, and deficits in antioxidant enzyme activities in the hippocampus, which were attenuated by morin supplementation. VPA-induced increases in neuroinflammatory markers and decreases in brain derived neurotrophic factor (BDNF) levels were reversed by morin treatment. Prenatal morin supplementation also exhibited neuroprotection against VPA-induced hippocampal neuronal damages, as seen in the preserved hippocampal neural architecture of the morin-treated groups. These findings showed that prenatal administration of morin prevented valproic acid-induced autistic-like behaviour in the offspring of WWE. This could be attributed to the augmentation of the oxido-inflammatory system and neuro-protective defence mechanisms via upregulation of BDNF in the brain.

Keywords

Morin, Anti-epileptic drugs, Valproic acid, Autism, Women with epilepsy, Pentylenetetrazol

Correspondence: Vivian O. Ojiakor, PhD, Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, College of Medicine, Parklane. Enugu State, Nigeria E-mail: vivianatuadu@gmail.com; Phone Number: +2348033231118; ORCID: 0000-0002-9717-2911

Chike I.P Anibeze- 0000-0002-9585-5356, chike.anibeze@esut.edu.ng; Ignatus I. Ozor: 0000-0002-9071-735X, Ikemozor@yahoo.com

Cite as: *Anibeze, C.I.P., Ojiakor, V.O. and Ozor, I. I. (2024). Evaluation of the therapeutic potential of prenatal morin supplementation on valproic acid-induced autism in offspring of epileptic pregnant Wistar rats. Nig. J. Neurosci. 15(2):* 48-61. *https://doi.org/10.47081/njn2024.15.2/002*

INTRODUCTION

About 60 million people worldwide suffer from epilepsy, half of whom are women. About one-third of women with epilepsy (WWE) are of childbearing age, and the incidence of seizures increases by 17–37% during pregnancy (Klein, 2014; Błaszczyk *et al*., 2022). WWE experience challenges throughout their reproductive lives, which place them and their unborn offspring at risk of adverse effects (Nucera *et al*., 2022). Epilepsy and anti-seizure medicines may affect not only the woman with epilepsy but the physical development, brain functioning, and even behaviour of their offspring in adult life. Both seizure and anti-seizure medicines used in the management of seizures are potentially dangerous to the foetus (Nucera *et al*., 2022). Therefore, an

epileptologist managing the WWE is burdened with the responsibility of weighing the teratogenic risks of a given anti-seizure medicine against seizure-associated risks for mother and child during pregnancy.

The effect of seizures on the foetus has been extensively studied (Laganà *et al*., 2016; Vlasov, 2023). Seizures probably lead to a decrease in blood flow to the placenta, miscarriage, epilepsy in offspring, and developmental delays. If a woman has repeated seizure episodes during pregnancy, aside from the potential for physical injury, the repeated episodes of lack of blood flow might restrict foetal growth (Klein, 2014). Hence, most patients require chronic administration of anti-epileptic drugs (AEDs) to manage their seizure episodes during pregnancy (Laganà *et al*., 2016). The offspring of women who took AEDs while pregnant had morphological defects, as AEDs have been shown to affect children's physical and neurodevelopmental functioning and even behaviour in adult life (Tomson, 2005; Veiby *et al*., 2013). Valproic acid (VPA) is one of the major AEDs, and it carries a major risk for neurocognitive impairment in children, besides its risk for minor congenital malformations. Children exposed to valproate exhibit a reduced intelligence quotient, memory, attention, or language skills compared with non-exposed children. It carries a significant risk for autism (Veroniki *et al*., 2017), as research has linked prenatal VPA exposure and ASD in humans and animal models (Roullet *et al*., 2013).

Autism is a broad term used to describe a group of complex neuro-developmental disorders characterised by impairments in social communication and interaction, restricted or repetitive behaviours, and learning impairments (American Psychiatric Association, 2013). Autism is also referred to as an autism spectrum disorder (ASD), as it can manifest differently in each person: any given individual is likely to show some, but not all, of the characteristics associated with it and may exhibit them to varying degrees (Wing *et al*., 2011). ASD has multiple mechanisms of action, including exacerbated oxidative stress and neuroinflammation, resulting in defective neuronal synapses, altered neurotransmitter homeostasis, and different neurotropic factors in the brain (Johannessen, 2000; Chugani, 2011; Petrenko *et al*., 2014). *In vitro*, valproate leads to deoxyribonucleic acid (DNA) fragmentation or gene expression dysfunction, pointing to apoptosis, which is a result of an imbalance in the antioxidant defence system of the body (Petrenko *et al*., 2014; Veroniki *et al*., 2017).

Autism is mainly associated with both genetic and environmental factors, including rare gene and/or DNA mutations as a result of interactions between multiple genes in different loci and environmental toxins (Rylaarsdam and Guemez-Gamboa, 2019; Lin *et al*., 2023). Exposure to toxins during embryogenesis could affect brain development and cause neuro-developmental disorders (Lyall *et al*., 2014). Rats treated with VPA *in utero* demonstrated delayed maturation, delayed motor development, and locomotor and repetitive, stereotypic-like hyperactivity combined with lower exploratory activity, a reduced number of social behaviours, and increased latency to social behaviours, which are all features of autism (Schneider and Przewłocki, 2005; Schneider *et al*., 2008). Furthermore, a study of the use of VPA during pregnancy showed a higher risk of autism-like disorder in the offspring of VPA-treated women compared with the use of other anticonvulsants (Schneider *et al*., 2005). However, despite all the downsides of VPA, it is one of the AEDs with the best treatment outcome in WWE (Błaszczyk *et al*., 2022). Its use is further reinforced by its efficacy and high dependency rate on other alternative antiepileptic drugs (Laganà *et al*., 2016). Hence, there is a need for the development of a therapeutic intervention that will enable the beneficial effects of VPA, while preventing its adverse effects on the offspring of these WWE.

Some interventions, such as supplementation with bioflavonoids, breastfeeding, and postpartum stress reduction, may improve neurodevelopmental outcomes in WWE before and during pregnancy (Veiby *et al*., 2013), suggesting that appropriate interventions might improve the outcomes of pregnancies where there is a risk of developing ASD (Nucera *et al*., 2022). Bioflavonoids are constituents of medicinal plants used as herbal medicines in traditional medical practice and are considered valuable therapeutic agents in modern medicines. Morin (3, 5, 7, 2, 4-pentahydroxyflavone) is a naturally occurring non-toxic flavonoid isolated from members of the Moraceae family such as onion and mulberry figs (Fang *et al*., 2003). An extensive literature survey reveals that morin possesses a wide range of pharmacological properties, including antioxidant (Sreedharan *et al*., 2009), anti-inflammatory (Kapoor and Kakkar, 2012), neuromodulatory (Merwid-Lad, 2012), chemoprotective (Kawabatar *et al*., 1999), and neuroprotective activities (Ponou *et al*., 2010; Wang *et al*., 2013).

Several psychiatric and neurodegenerative disorders, including schizophrenia, depression, Alzheimer's disease, Parkinson's disease, and autism, have been linked to increased oxidative stress as well as increased neuroinflammation. Since oxidative and inflammatory stress are considered potent intracellular signalling mechanisms that induce changes in the brain (Pazvantoglu *et al*., 2009), research has discovered that bioflavonoids improve health conditions by many mechanisms, which include reduction of oxidation and inflammation (Akinluyi *et al*., 2020), regulation of neurotransmitters, and upregulation of neurotrophic factors, thereby protecting the neurons from permanent injuries (Nijveldt *et al*., 2001). Morin administration has been shown to improve cognitive function in mice by attenuating lipid peroxidation (Fang *et al*., 2003; Chen *et al*., 2017). Many studies have suggested that Morin exhibits antioxidant and anti-inflammatory effects (Galvez *et al*., 2001; Subash and Subramanian, 2009; Akinluyi *et al*., 2020), hence blocking damaging effects in the offspring of pregnant Wistar rats. There are currently no pharmacological treatments approved by the Food and Drug Administration for the core impairments of ASD (Posey *et al*., 2008). Rather, the currently approved medications to treat ASD principally target the accessory traits of irritability, aggressiveness, and repetitiveness (McPheeters *et al*., 2011). Developing appropriate pharmacological treatments is further complicated by the high prevalence of comorbid conditions in ASD, including affective disorders (such as

anxiety and/or depression) or neurological disorders (such as epilepsy), often requiring additional prescriptions (Morgan *et al*., 2003; Bauman *et al*., 2010). Therefore, there exists a need to develop effective pharmacological interventions capable of preventing the development of the core neurobehavioural impairments in ASD. Hence, this study seeks a suitable bioflavonoid with the potential to putatively cushion the negative effects of VPA on the offspring of WWE while giving the women the best relief from seizure episodes.

MATERIALS AND METHODS

Experimental Animals and Ethical Approval

The experiment was carried out on the offspring of adult female Wistar rats weighing 180–220 g (n = 30). The female rats were housed at the Enugu State University of Science and Technology (ESUT) animal holding facility in standard cages throughout the treatment period, under suitable conditions of 28°C, 50% humidity, and 12-hour light/12-h dark cycles. The rats were acclimatised to the environment for two weeks prior to experimental use. They were allowed free access to clean water and standard livestock pellets (Guinea Feed Nigeria Limited) *ad libitum.* The experimental procedure was approved by the Ethics Committee of Enugu State University College of Medicine (ESUCOM), with the number: ESUCOM/FBMS/ETR/2022/004, and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals NIH Publication (NIH, 1985).

Experimental Procedure

The kindling of the female rats involved a single dose of 40 mg/kg pentylenetetrazol (PTZ) (Sigma, USA) dissolved in 1 mL of normal saline (0.9%) intraperitoneally (i.p.), every 48 h. A total of seven doses of PTZ were given to each rat, and convulsive behaviour was observed for 30 min after each PTZ injection. This kindling process was done before mating. The rats were allowed to mate, which was done 14 days after for kindling rats. Male rats were introduced into the cages overnight for possible mating. Vaginal smears were examined for a seminal plug as evidence of mating. The day of plug detection was designated gestational day (GND) 0. In the absence of a seminal plug, mating was repeated. Upon confirmation of pregnancy, the female rats were randomly separated into 5 groups of 6 rats each. Rats that were not kindled before mating were the control group (non-kindled normal control) and received 1 mL of normal saline, while the kindled rats were separated into four treatment groups as follows: PTZ (40 mg/kg i.p.), PTZ + VPA (75 mg/kg i.p.), PTZ + VPA + Morin (25 mg/kg i.p.), and PTZ + VPA + folic acid (20 mg/kg i.p.). Following confirmation of pregnancy and grouping, treatments were carried out on GND 8-18. A sub-convulsive dose of PTZ was administered every alternate day in order to sustain kindling during the pregnancy period, while the VPA dose of 75 mg/kg i.p. was chosen according to the VPA dose that completely prevents PTZ-induced seizures within 45 min after i.p. injection of PTZ in our pilot study. Morin (25 mg/kg) and folic acid (20 mg/kg) doses were adopted from Ben-Azu *et al*. (2018) and Nagy *et al*. (2020), respectively. On the GND 19, animals were individually housed in preparation for delivery. For those female rats who did not give birth after the GND 23 day, the pregnancy was considered a failure. There were at least two litters from each female, and as such, two litters were randomly selected from each female, making it 12 pups in each group.

Pups were separated from their mothers on postnatal day (PND) 28. On PND 32, social behaviour, spatial working memory and repetitive behaviour, and anxiety were assessed in the pups using the three-chamber social assay, Y-maze, and elevated plus maze tests (EPM), respectively. On PND 45, the offspring were euthanized and brains removed for biochemical, histological, and immunohistochemical assays. All drug treatments and behavioural tests were performed between 9:00 a.m. and 2:00 p.m. to prevent the influence of hormonal fluctuations (Fig. 1).

Behavioural Test

Six pups were randomly selected per group for behavioural studies (Li *et al*., 2018) on PND 42-45. The pups were handled prior to the start of the behavioural experiments to prevent the influence of fear on the experimental results; the experimenters handled the mice for five minutes per day for three consecutive days to reduce fear.

Assessment of Repetitive Behaviour and Spontaneous Alternation in Y-Maze

The Y-maze was employed on postnatal day (PND) 42 to evaluate spontaneous alternation. Spontaneous alternation in the Y-maze served as an indicator of both repetitive behaviour and memory function. To assess spontaneous alternation in a Y-maze, the method described by Merali *et al*. (2014) was used. The Y- maze is a Y-shaped apparatus with three equal gray-coloured, opaque arms; 40 × 12 × 30 cm, orientated at 120^{o} angles from each other. Each arm was marked with letters A, B, and C. Each rat was introduced at the end of one arm of the Y-maze and was allowed to explore the arms freely over a 6 min period (1 min for arena acclimatization). The series of arm entries was recorded to determine the number of spontaneous alternations. An entry occurs when all four limbs of the rat are within an arm. Correct spontaneous alternation is defined as entry into all three arms consecutively, in 5 min. The percentage alternation was calculated as {(number of alternations /total number of entries-2) × 100}.

Assessment of Anxiety in Elevated Plus Maze

The elevated plus maze (EPM) was used to assess anxiety behaviour in rats at PND 43. The elevated plus-maze was made of plywood and consisted of two opposite open and close arms (50 ×10 × 40 cm), elevated to a height of 50 cm. The animal was placed in the central portion of the EPM and allowed to acclimatise for 1 min. After habituation to the EPM, the time spent and number of entries into the open and closed arms in 5 min were recorded by an observer unaware of the treatment groups. An entry was de-

fined as entering into one of the arms with all four paws (Kraeuter *et al*., 2019).

Assessment of Social Interaction in a Three Chamber Test

Social interaction tasks in the form of sociability and social preference were assessed in rats during puberty (PND 44–45) using the three chamber apparatus (Rein *et al*., 2020). The three-chamber apparatus consisted of a Plexiglas box (70 × 70 × 50 cm) divided into three identical communicating compartments. The social interaction testing occurs in three sessions; the first section is the habituation period. A test rat was placed in the empty central compartment of the testing box and allowed to acclimatise for 5 min. At this stage, the doors between compartments were closed. In the second session, a novel rat was introduced in a wire cage in the left compartment (stranger zone 1), while the right compartment contained an empty wire cage for the sociability test. The rat in the central compartment was allowed to freely explore the two compartments after opening the doors, allowing the test rat the opportunity to encounter a never-before-met intruder under one wire cage and an empty wire cage. The number of crosses from one compartment to another and time spent in each compartment were recorded for 10 min.

In the 3rd stage (social novelty preference test), the test rat then encounters the first intruder as well as a second never-before-met intruder under another wire cage in the "social novelty" session. The test rat was confined to the central compartment, and a second unfamiliar rat (age and sex matched to the experimental rat; stranger zone 2) was placed in the previously empty wire cage of the sociability test. The time spent by the experimental rat in both side chambers was recorded for 10 min. The time spent sniffing wire cage, the time spent in each chamber, and the number of entries into each chamber are recorded.

Sociability and social novelty preference were expressed as sociability and social novelty preference index, respectively. The sociability index is calculated as the ratio of time spent by the test animal on the stranger side to the time spent by the test animal on the empty side, while the social novelty preference index is calculated as the ratio of time spent by the test animal on the novel side (stranger zone 2) to the time spent on the familiar side (stranger zone 1) (Kumar and Sharma, 2016; Rein *et al*., 2020).

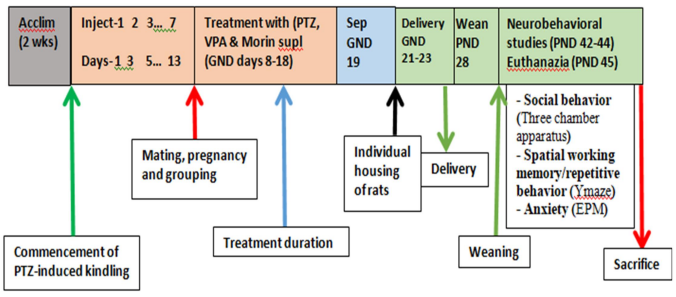


Fig. 1: Diagrammatic representation of the experimental design and drug treatments of the adult pregnant female rats

Animal Sacrifice and Tissue Collection

Six animals meant for histological and immunohistochemical examinations were anaesthetized with ketamine (7.5 mg/kg) and xylazine (1 mg/kg), while six of those meant for biochemical and neurochemical assays were euthanized via cervical dislocation. This is to avoid interactions of the drug of anaesthesia with biochemical molecules. All the brain samples were perfused transcardially with normal saline, followed by a 0.1 M phosphate-buffered saline solution. The whole brain was quickly harvested after perfusion, and the hippocampus was dissected out on an ice-cold plate. The isolated hippocampus was weighed and fixed in 4% paraformaldehyde-phosphate buffered saline (PBS) for 48 h.

Tissues meant for biochemical assays were not fixed but homogenised fresh in 0.03 M sodium phosphate buffer, pH-7.4 with an Ultra-Turrax T25 (USA) homogenizer at a speed of 9,500 rpm. The hippocampal homogenates were kept at -20 °C and subsequently processed as described under the neurotransmitters and enzymes assay methods section.

Histology

Sections for histology were then dehydrated, cleared, paraffin wax embedded, and sectioned using a Cambridge rocker microtome at 5 µm. Sections on slides were processed and stained with haematoxylin and eosin for histoarchitectural organisation of the hippocampus and cresyl fast violet stain for Nissl substance. Hippocampal tissue structures were observed histologically for signs of neuronal damage in Nissl stained and H & E sections.

Immunohistochemical Staining for Brain Derived Neurotrophic Factor (BDNF)

BDNF immunohistochemistry was performed on hippocampal tissue sections following the manufacturer's kit instructions and the modified method of Edelstein *et al*. (2014). The brain sections were briefly subjected to the process of deparaffinization and hydration using xylene and graded alcohols (100, 90, and 80%) for 5 min each. Additionally, 10% hydrogen peroxide (H_2O_2) was added with methanol for treatment (5 min) to protect against the activity of endogenous peroxidase. Thereafter, tissue sections were rinsed with distilled water, placed in a citrate buffer tank, and heated in a water bath for 5 min for antigen retrieval. Slides were washed thrice with PBS containing 0.02% Tween 20, before adding protein blocking solution for 10 min at room temperature (25 °C). Tissue sections were incubated with a primary antibody [(mouse monoclonal anti-BDNF antibody), abeam (EPR1292), ab205067] (1:300) for 2 h at room temperature. After 5 min of PBS-Tris wash (thrice), the sections were incubated with rabbit polyclonal secondary antibody (Abcam) at room temperature for 30 min. DAB (3,3′-diaminobenzidine) reagent was added to the tissue sections and allowed to incubate for 6-10 min at room temperature before washing with PBS five times and with distilled water. Then, slides were incubated with haematoxylin for 60 s, rinsed with distilled water, and allowed to drain before mounting with distrene plasticiser xylene.

Measurement of Oxidative Stress-Related Indicators

Hippocampal tissues from ipsilateral sides of the six rats in each group were weighed, and deionized water was added at a ratio of 1:9, after which the samples were homogenised in an ice bath. The samples were centrifuged for 15 min at 4°C, and the supernatant was collected. Reactive nitrogen species (RNS) nitric oxide (NO) and malondialdehyde (MDA) contents, as well as superoxide dismutase (SOD) activity, catalase (CAT) activity, and glutathione (GSH) activities in hippocampal tissue, were measured with different commercial assay kits (Jian Cheng Bioengineering Institute, China) following the manufacturers' protocols for each kit.

Detection of Neurotransmitters and Inflammatory Cytokines in the Hippocampal Tissue

Brain tissue samples from six rats in each group were used to measure the expression levels of the pro-inflammatory cytokines interleukin-1 (IL-1*β*) and tumour necrosis factor-alpha (TNF-*α*), and neurotransmitters such as serotonin (5-HT), dopamine (DA), and adrenaline using enzyme-linked immunosorbent assay (ELISA) kits (Boster Biological Engineering Co., Ltd., China, and Jian Cheng Bioengineering Institute, China) following the manufacturers' protocols for each kit. The data obtained were expressed as ng/g tissues. Experiments were performed at least three times.

Statistical Analyses

The Graphpad Prism software version 7 was used for data processing and analysis. All data are presented as the mean ± standard error of mean (SEM). The results were statistically assessed by one-way ANOVA, followed by Tukey's post hoc multiple test for comparison between experimental groups, and the statistical significance was set at $p < 0.05$.

RESULTS

Effects of Morin Supplementation on the Social Behaviour of VPA-Induced Autism in Offspring of Epileptic Pregnant Rats

In-utero VPA exposure caused significant reduction in the time spent in the stranger chamber, sociability index and social novelty preference index in three chambered apparatus as compared to the control. There was no significant difference in the time that rats spent exploring the left and right chambers during the habituation phase, indicating that rats in all groups had no preference for any chamber (Fig. 2a-d).

Sociability and sociability index

Rats across treatment groups were allowed to explore new environment in the three chamber apparatus. The control group rats spent significantly more time in the chamber with unfamiliar rat ($P<0.001$; 381.2±19.22) compared to rats in PTZ+VPA group (86.6 ± 10.70). However, in-utero VPA treatment resulted in animals spending more significant time ($P<0.001$, 395.6±32.37) in the empty chamber than in the unfamiliar rat chamber (86.6±13.07), (Fig. 2a). This indicates reduced sociability. Furthermore, Morin administration produced significant increase in time ($P<0.05$; 402±3.03) spent with unfamiliar rat as compared to time spent in the empty chamber. Therefore, Morin administration significantly prevented VPA-induced deficit in sociability index.

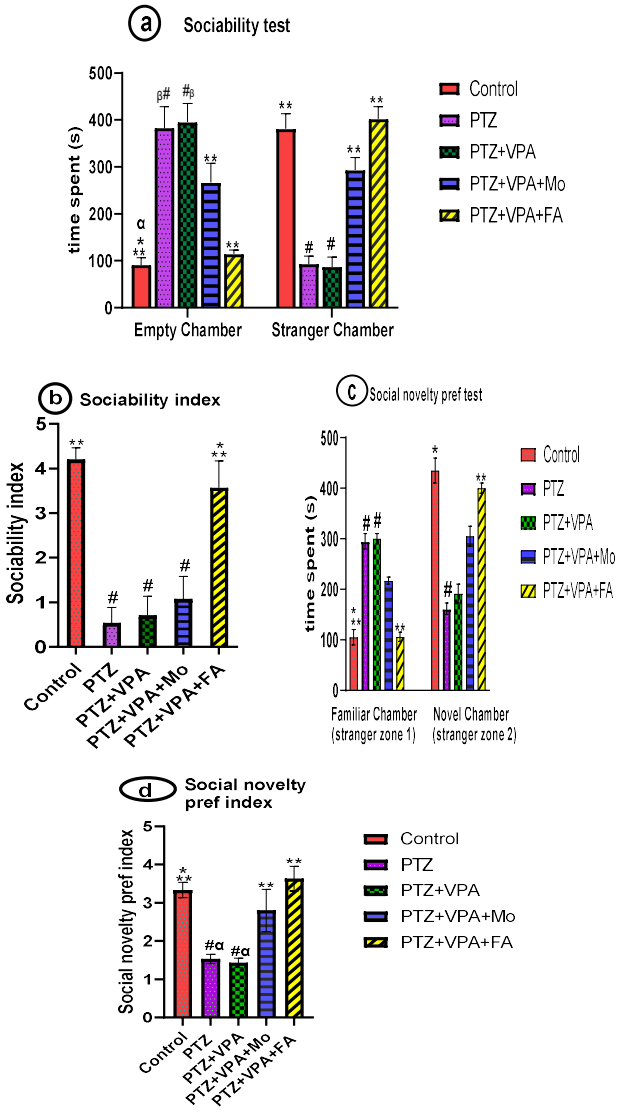


Fig 2 (a-d): Effect of Morin supplementation on (A) Time spent in empty chamber and stranger chamber (B) Time spent in familiar chamber and novel chamber (C) Sociability index (D) social novelty preference index in three chamber sociability test. Values are expressed as mean ±S.E.M (n=6). # = $p < 0.05$ versus control treated; *= $p < 0.05$ versus PTZ treated; **= $p < 0.05$ versus PTZ+ VPA treated; α = $p < 0.05$ versus Morin treated; β= $P<0.05$ versus folic acid treated.

Social Novelty Preference Test and Social Novelty Preference Index

Rodents are social animals and will prefer to spend time with unfamiliar rats than an already familiar one. The control animals spent significantly more time ($p < 0.05$;

415.6±28.52 s) in the chamber with the unfamiliar rat (stranger zone 2) compared to the time spent (126.8±17.86 s) with familiar rat (stranger zone 1) in the three chamber apparatus. This is an evidence of social memory formation. Post hoc analysis revealed that VPA-treated group spent significant more time (P<0.05, 300.5±15.80) in the familiar rat chamber (stranger zone 1) and a reduction in time (191.0±9.24), spent with the novel rat in (stranger zone 2) which indicates social memory deficit. However, administration of Morin significantly prevents VPA-induced reduction in time spent in novel rat chamber (stranger zone 2) (P<0.05, 400.15±13.80) and significantly decreased time spent in the familiar chamber (stranger zone 1); (P<0.05, 106.7±15.10), (Fig. 2c-d). Prenatal administration of VPA and PTZ significantly reduced social novelty preference index (P<0.05; 1.32±0.10), when compared with the control animals (3.17±0.41); (Fig. 2d). VPA-induced deficit in social novelty preference index was significantly reversed by morin administration.

Morin supplementation overturns memory deficit and repetitive behaviour in VPA-induced autism in offspring of epileptic Wistar rats in the Y-Maze

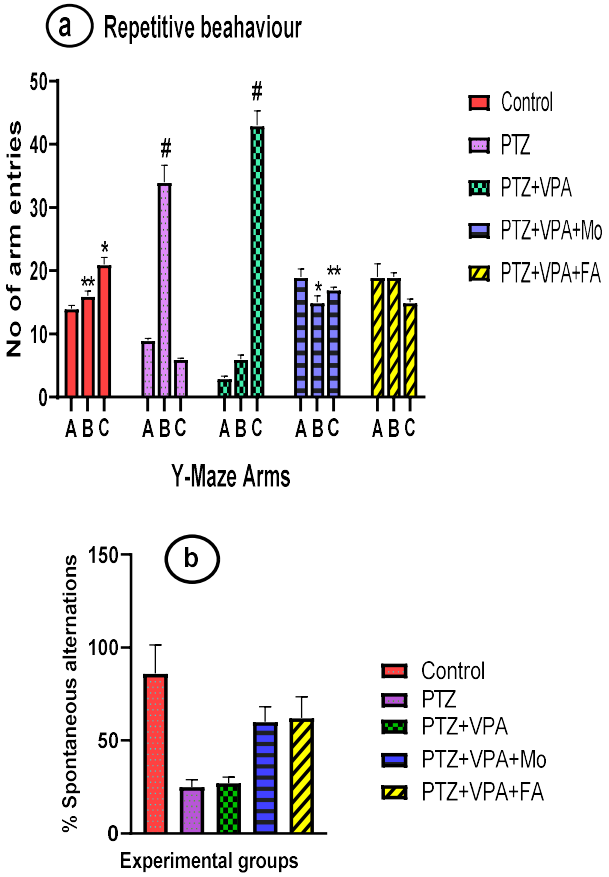
The result shows that in-utero VPA and VPA+PTZ administration significantly increased repetitive behaviour, evidenced by the increased entry into a particular arm as compared with the normal control group having almost equal distribution of entries in all arm. The supplementation with Morin and folic acid also decreased repetitive behaviour, as seen in the PTZ+VPA+Mo and PTZ+VPA+FA groups. These changes are seen to be statistically significant when compared with the control group.

Effect on Learning and Memory Impairment

Memory impairment is one of the significant symptoms of autism. In the present study, the role of Morin supplementation on learning and memory impairment in animals with VPA-induced autism (memory impairment) was characterized using the Y-maze (3b). The rat groups treated with Morin or folic acid showed an enhancement in the memory indices when compared to the VPA and VPA+PTZ groups. This is evidenced by the high percentage of correct spontaneous alternation in the control and Morin groups as compared to the VPA and VPA+PTZ groups. Comparatively, PTZ+VPA+Mo group showed a higher % spontaneous alternation than either of VPA or PTZ+VPA groups.

Anxiolytic effects of Morin against VPA-mediated anxiogenicity in VPA-induced autism in offspring of epileptic Wistar rats in EPM

According to the result in figure 4, VPA-induced some anxiety-like behaviour in the offspring of epileptic Wistar rats when exposed to the EPM behavioural paradigm. The VPA group showed a substantially decreased open arm duration even though the open arm entry was not significantly different when compared to the control group. When combined, as seen in the PTZ+VPA+Mo and PTZ+VPA+FA groups, there was an increase in the open arm entry and duration, relative to the VPA group.



A= Arm A, B=Arm B, C= Arm C

Fig 3 (a-b): Effect of Morin supplementation on repetitive behaviour (a) and % spontaneous alternation (b) in Y-maze test. Values are expressed as mean±S.E.M (n=6). #P < 0.05 versus control treated; *P < 0.05 versus VPA treated animals; $_{\beta}$P<0.001 versus Morin treated. Statistical analysis was by one way ANOVA followed by Tukey's post hoc multiple comparison tests.

Effects of Morin intervention on oxidative and nitrosative stress in the hippocampal tissue of VPA-induced autism in offspring of epileptic Wistar rats

Lipid peroxidation is believed to be involved in neuronal damage in experimental animals. The effect of Morin supplementation on oxidative and nitrosative stress in the hippocampus is shown in Figure 5(a-e). The reactive nitrogen species (NO) and the by-product of lipid peroxidation; malondialdehyde (MDA) contents in hippocampal tissue from rats in the PTZ and PTZ+VPA group were significantly higher than those in hippocampal tissue from rats in the PTZ+VPA+Mo group (P<0.05). Notably, NO and MDA contents significantly decreased after Morin intervention (P<0.05). SOD activity, CAT activity and antioxidant GSH levels in hippocampal tissue from rats in the PTZ and PTZ+VPA groups were significantly lower than those in hippocampal tissue from rats in the control and Morin groups (P<0.05). The above mentioned antioxidant enzyme activities and GSH levels significantly increased after Morin supplementation (P<0.05). There was no significant

difference in these levels between rats in the Morin and folic acid supplementation groups (P>0.05). These results suggest that Morin intervention was able to reduce oxidative stress and protects against oxidative stress injury in the brain of autistic rats.

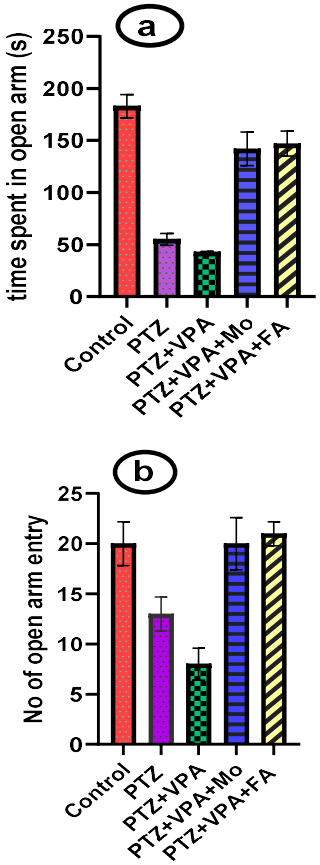


Fig 4 (a-b): Effect of Morin supplementation on (a) Time spent in open arm (Open arm duration; OAD) (b) Number of open arm entries (OAE) in elevated plus maze test (EPM). Values are expressed as mean±S.E.M (n=6). #P < 0.05 versus control treated; *P < 0.05 versus PTZ treated animals; **P < 0.05 versus PTZ+ VPA treated; αP<0.001 versus Morin treated; βP<0.005 versus folic acid treated. Statistical analysis was by one way ANOVA followed by Tukey's post hoc multiple comparison tests.

Effects of Morin Supplementation on Neuroinflammation in the Hippocampal Tissues in VPA-induced Autism in Offspring of Epileptic Wistar Rats

To examine the effect of and Morin supplementation on the inflammatory response in VPA-induced autism in the offspring of epileptic rats, the levels of inflammatory markers in the hippocampal tissue of all rats were examined. As shown in Figure 6, the levels of inflammatory cytokines IL-1β and TNF-α in the hippocampal tissue of PTZ+VPA group were significantly higher than those of rats in the control group (all P<0.05). Morin supplementation significantly decreased the expression of IL-1β and TNF-α in the hippocampal tissue (P<0.05), and there was no significant difference in the levels of inflammatory cytokines between rats in the Morin and folic acid (P>0.05) groups. These results suggest that Morin intervention reduces inflammation in autistic rats.

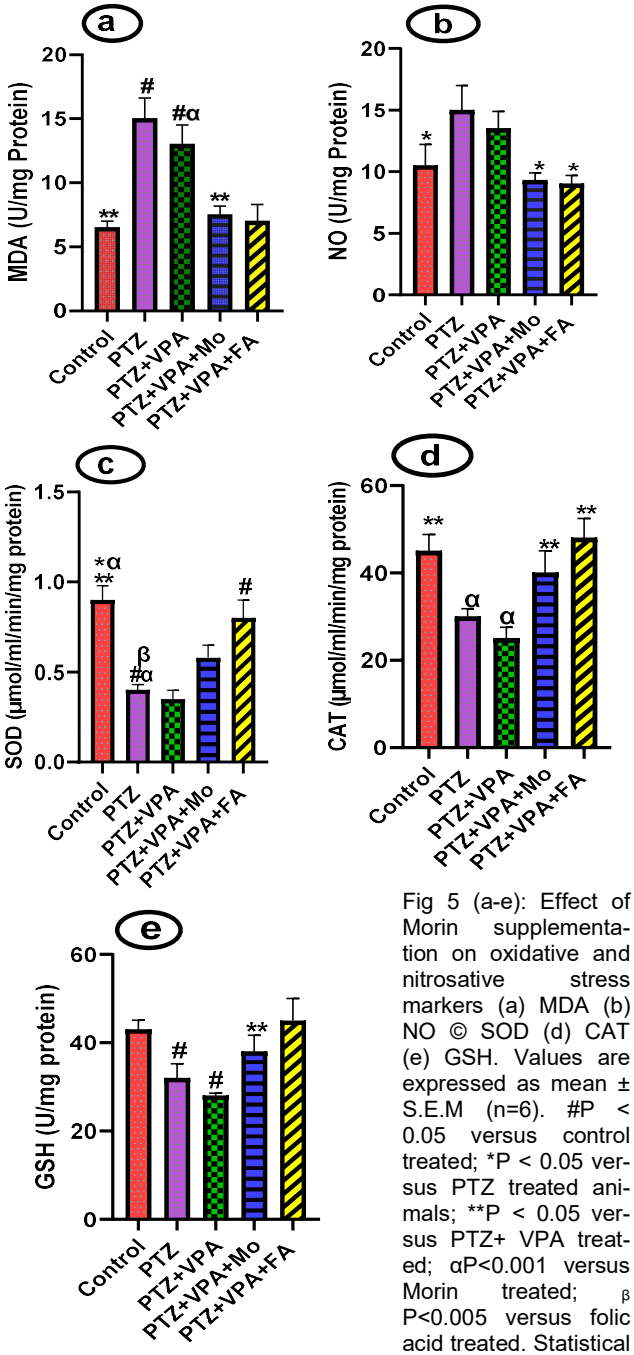


Fig 5 (a-e): Effect of Morin supplementation on oxidative and nitrosative stress markers (a) MDA (b) NO © SOD (d) CAT (e) GSH. Values are expressed as mean ± S.E.M (n=6). #P < 0.05 versus control treated; *P < 0.05 versus PTZ treated animals; **P < 0.05 versus PTZ+ VPA treated; αP<0.001 versus Morin treated; β P<0.005 versus folic acid treated. Statistical analysis was by one way ANOVA followed by Tukey's post hoc multiple comparison tests

Changes in Hippocampal Monoamine Neurotransmitter Levels following Morin Supplementation in VPA-Induced Autism in Offspring of Epileptic Wistar Rats

Regarding hippocampal monoamine neurotransmitter levels (Fig. 7), PTZ and VPA treatment significantly increased

Dopamine level compared with control while PTZ and VPA treatment significantly decreased adrenaline levels compared to the control and Morin treated groups. Serotonin level was also decreased by PTZ+VPA but the decrease was not statistically significant.

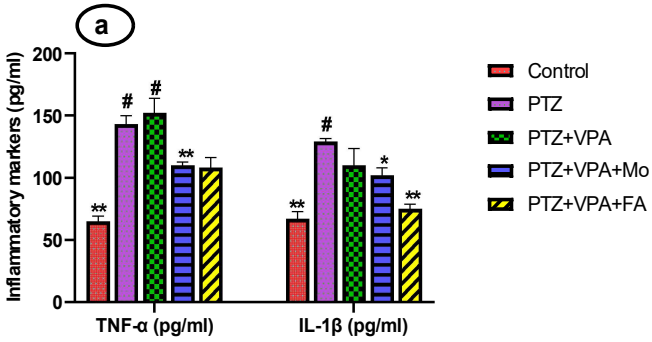


Fig 6 (a): Effect of Morin supplementation on neuroinflammatory markers TNF-alpha and IL-1beta. Values are expressed as mean ± S.E.M (n=6). #P < 0.05 versus control treated; *P < 0.05 versus PTZ treated animals; **P < 0.05 versus PTZ+ VPA treated; αP<0.001 versus Morin treated; β P<0.005 versus folic acid treated. Statistical analysis was by one way ANOVA followed by Tukey's post hoc multiple comparison tests

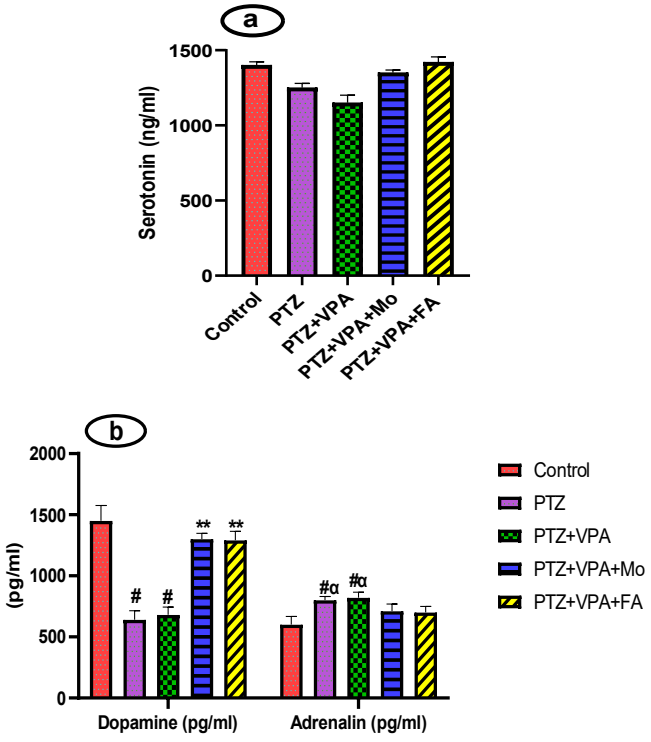


Fig 7 (a-b): Effect of Morin supplementation on monoamine neurotransmitters (a) Serotonin (b) Dopamine and Adrenalin. Values are expressed as mean ± S.E.M (n=6). #P < 0.05 versus control treated; *P < 0.05 versus PTZ treated animals; **P < 0.05 versus PTZ+ VPA treated; αP<0.001 versus Morin treated; β P<0.005 versus folic acid treated. Statistical analysis was by one way ANOVA followed by Tukey's post hoc multiple comparison tests

Effects of Morin supplementation in neural cell integrity, Nissl substance and BDNF expression in VPA-induced autism in offspring of epileptic Wistar rats

Specifically, the CA1 hippocampal area of rats in the PTZ and PTZ+VPA groups revealed; highly compromised neural integrity, sparsely distributed and irregularly arranged neural cells, with neuronal pyknosis in the H&E stains, a decrease in the number of Nissl bodies and increased chromatolytic cells in the CFV staining and a decreases in the expressions of BDNF positive cells in the BDNF staining. In offspring of rats in the control, Morin and folic acid groups, the cell morphology of the hippocampal area was relatively normal, complete and evenly populated, cells were arranged neatly, with nicely shaped pyramidal neurons in the H&E stains. There was reduced number of chromatolytic cells and normal Nissl expression in the CFV stain and finally, there was increased expression of BDNF positive reactive cells as compared to the PTZ+VPA group. There was no observable difference in the hippocampal area between offspring of rats in the control group and the Morin or folic acid groups.

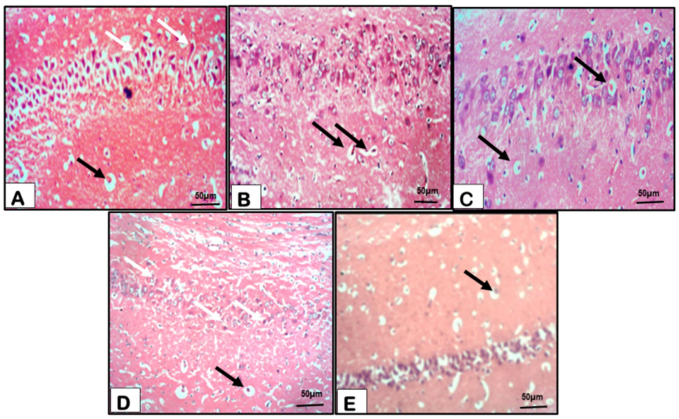


Fig. 8 (A-E): Representative photomicrographs of CA1 area of the hippocampus of Wistar rat brain stained with H&E. The Control (A) revealed normal histological cytoarchiture and normal neural population; The PTZ (B) showed the presence of dark chromatin nuclei and decrease neural population with pyknotic cells. The PTZ+VPA (C), revealed pyknotic neurons. The PTZ+VPA+Mo and PTZ+VPA+FA (D and E, respectively) revealed increased normal population of nuclei with few pyknotic neurons. White arrow– normal neuron; Black arrow - pyknotic neurons. X400; Scale bar =50µm

DISCUSSION

Animal models used for the study of autism mimic the core behavioural manifestation in autism including memory loss, anxiety, hyperactivity, repetitive behaviours and social aversion (Rylaarsdam and Guemez-Gamboa, 2019). VPA is reported as a neurodevelopmental toxin, having been associated with neurobehavioral deficits in experiment animals (Petrenko *et al*., 2014). In this study, in-utero Morin supplementation significantly attenuated VPA-induced anxiety in the rats, as evidenced in the significantly increased time duration in the open arm of the elevated plus maze.

This could be attributed to the anxiogenic property of Morin. . In addition, Morin supplementation also significantly alleviates VPA-induced memory deficit. This observation could be attributed to the ability of Morin to inhibit behavioural hyperactivity as demonstrated in previous studies (Schneider *et al*., 2006; Ben-Azu *et al*., 2017; Cezar *et al*., 2018; Wu *et al*., 2022).

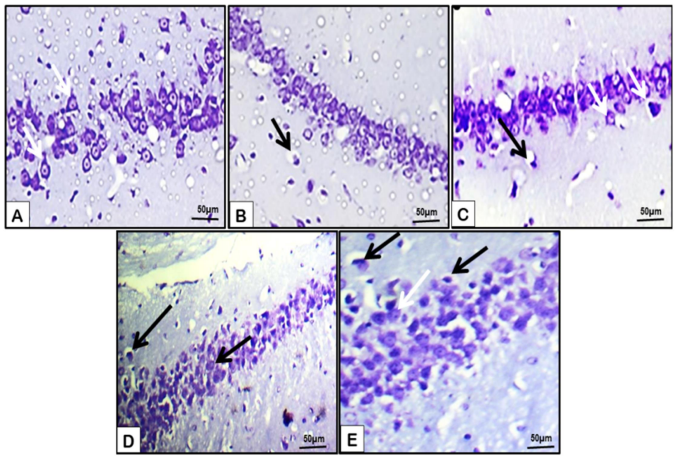


Fig. 9 (A-E): Representative photomicrographs of CA1 area of the hippocampus of rat brain stained with Crysl fast violet (CFV): Control slide A, showed evenly distributed and well stained Nissl bodies (NB). The PTZ and PTZ+VPA slides (B-C) presented with varying degrees of central (CC) and peripheral chromatolysis (PC). The PTZ+VPA+Mo and PTZ+VPA+FA slides (D-E), showed normal staining and relatively evenly distributed Nissl bodies. White arrow– normally stained neuron; Black arrow - central or peripheral chromatolytic neurons. X400; Scale bars = 50µm

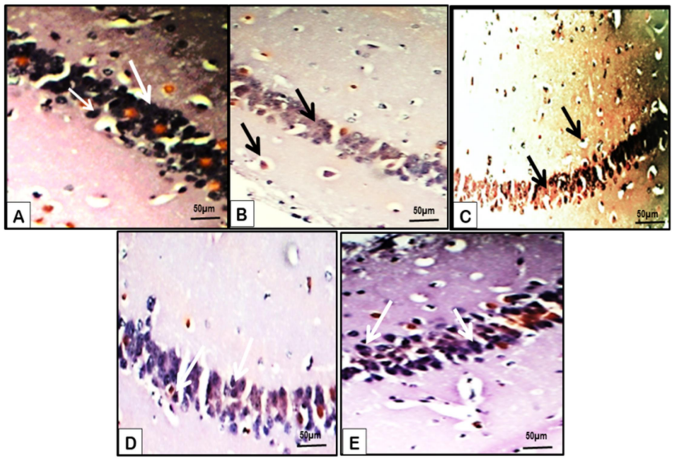


Fig. 10 (A-E): Representative photomicrographs of CA1 area of the hippocampus of rat brain labelled for BDNF expression: The control slide A, revealed high immunopositive cell expression of BDNF immunopositive cells; slides PTZ and PTZ+VPA (B-C), showed low immunopositive cell expression of BDNF while slides PTZ+VPA+Mo and PTZ+VPA+FA (D-E), showed high immunopositive cell expression of BDNF. White arrow – high immunopositive cell expression of BDNF; Black arrow - low immunopositive cell expression of BDNF. X400; Scale bars = 50µm.

Repetitive patterns of behaviour, restricted interests and Memory impairment are key characteristics and symptoms of ASD (Wanger *et al*., 2006). Stereotyped behaviour which presents itself as continuous, ritualistic and functionless repetitive behaviour is one of the most prominent positive symptoms of autism, and it usually manifest in humans in the form of repetitive performance of a set of strange gestures or making the same kind of comments (Wang *et al*., 2015). In animal studies, it manifests itself as: repeated visit to the same arm in Y-maze paradigm, and can be assessed using the spontaneous alternation. A decrease in percentage spontaneous alternation was taken as an indicator of increased repetitive behaviour and impaired memory, while an increase in correct spontaneous alternation was taken as an indicator of decreased repetitive behaviour as well as an enhanced memory (Merali *et al*., 2014). In a study conducted by Heydari *et al*. (2021), VPA-exposed offspring recorded decreased spontaneous alternation suggesting increased repetitive behaviour or functional memory deficits in the Y-maze test. Additionally, a recent study affirmed that prenatal exposure to VPA significantly heightened repetitive behaviour in the Y-maze test (Ishola *et al*., 2020). In this regard, the findings in our study showed that the rate of spontaneous alteration significantly reduced in the rats with prenatal VPA exposure (PTZ+VPA group), which is suggestive of repetitive behaviour as well as spatial memory impairment in these animals and is in line with these previous studies.

Furthermore, another characteristic symptom of ASD is deficiency in social interaction. The three-chambered apparatus was used to assess social deficit in the experimental animals. The three-chamber test assesses cognition in the form of general sociability and interest in social novelty in rodent models of central nervous system disorders. Rodents normally prefer to spend more time with another rodent (sociability) and will investigate a novel intruder more than a familiar one (social novelty). Social novelty preference and sociability indices are used to assess the willingness of a rodent to socialize, as autistic individuals tend to shy away from socialization. In this study, Morin supplementation reversed VPA-induced social deficit as evidenced by the increased social novelty preference index in the Morin supplemented group (PTZ+VPA+Mo). Indeed, previous studies have reported similar findings (Ben-Azu *et al*., 2020). A decrease in time spent and number of entries into the open arm in elevated plus maze (EPM) test was considered as anxiety behaviour (Ishola *et al*., 2015). Anxiety-like behaviour is one of the hallmarks of autism. Our study recorded significant decreased time spent in the open arm by the VPA exposed rats but significant increase in time spent in the open arm by the Morin-supplemented group. This result is consistent with previous studies (Ben-Azu *et al*., 2020; Heydari et al., 2021).

Lipid peroxidation is believed to be involved in neuronal damage in experimental animals. Antioxidants such as SOD, CAT, GSH are essential in counterbalancing products of lipid peroxidation, production of free radicals such as superoxide anion (O^{2-}), H_2O_2, hydroxyl radical (OH•), as well as nitric oxide (NO) and safeguard the cells against

oxidative damage (Sandhya *et al*., 2012; Ben-Azu *et al*., 2017). However, excessive production of these free radical may surpasses the cell's capacity of the antioxidants to neutralize the free radicals, ultimately lead to oxidative stress. MDA level is an important marker of oxidative stress, generated through the peroxidation of membrane lipids triggered by free radical production, leading to membrane damage and degradation (Ayala *et al*., 2014). Enhanced oxidative stress is one of the important pathological mechanisms of ASD (Usui *et al*., 2023), potentially leading to neural cell toxicity and DNA damage (Han and Chen, 2013), in the brains of ASD patients (Zhang *et al*., 2012; Manivasagam *et al*., 2020). Hippocampal tissue is one of the brain areas with high rate of oxygen consumption and is adversely affected in the event of oxidative stress as a result of high of ROS or RNS production and a decrease in the expression level of antioxidants in the brain causes irreversible oxidative stress injury in neurons (Manivasagam *et al*., 2020). Nonetheless, Morin as an antioxidant and free radical scavenger, is particularly important in maintaining redox homeostasis (Ben-Azu *et al*., 2017). This study revealed that Morin supplementation increased the expression of antioxidants (SOD, CAT and GSH) and decreased nitric oxide production and lipid peroxidation, which resulted in enhanced antioxidant capacity, reduced free radical production, and decreased oxidative stress in tissues and ultimately improves memory and social functions as recorded in the behavioural study. Previous finding had indicated that VPA induced oxidative stress because they increased the production of free radicals which in turn is associated with neural cell toxicity and DNA damage (Han and Chen, 2013). Bio-flavonoid antioxidants such as quercetin, apigenin, as well as Morin administered to experimental animals with neurodegenerative diseases has been documented to inhibited oxidative stress, thereby alleviating, neuronal damage and improving cognitive as well as social functions (Ben-Azu *et al*., 2017; Ogunlade *et al*., 2020; Bellavite, 2023).

The inflammation cascade has a vital role in the pathogenesis of neurodegenerative diseases (Trovato Salinaro *et al*. 2018), as neuroinflammation is one of the important pathogenic mechanisms of ASD. Neuroinflamation has been found in the hippocampal lobe of patients with ASD, manifested by the excessive increase in the levels of proinflammatory cytokines such as TNF-alpha and IL-1β (Matta *et al*., 2019). This study found that VPA increased neuroinflammation as seen by the elevated levels of the proinflammatory cytokines TNF-alpha and IL-1β, suggesting that VPA affects macrophage functions and stimulates the development of brain injury. This result aligns with past studies which reported neuroinflammation, following VPA in-utero exposure (Petrenko *et al*., 2014). However, morin supplementation inhibited the occurrence of neuroinflammation and reduced proinflammatory cytokine levels significantly, suggestive of its anti-inflammatory property (Akinluyi *et al*., 2020).

Neurotransmitter homeostasis, is essential for the healthy development of the brain, memory, motor function, and behaviour. Based on these insights, neurotransmitter system malfunction is hypothesized to be the root cause of ASD, impacting synaptogenesis, migration, and differentiation of neuronal cells, and ultimately the brain's developmental processes (Cetin *et al*., 2015). Neurochemical data have shown that monoamine neurotransmitters such as dopamine, 5-HT and adrenalin are associated with different symptoms of neurological diseases and their neuropathology (DiCarlo *et al*., 2019). In rodents, increased anxiety and hyperactivity has been linked to the ability of an agent to increased CNS excitatory neurotransmitter adrenaline, while social aversion has been linked to the ability of an agent to inhibit the neurotransmitter 5-HT (Chatterjee *et al*., 2015; Ogunlade *et al*., 2020). In this study, VPA exposure decreased DA and 5-HT while elevating adrenaline levels in the rats' hippocampus. Nonetheless, prenatal Morin supplementation was able to reverse the VPA-induced neurotransmitter alterations by increasing the levels of 5-HT and DA neurotransmitters while decreasing adrenaline level, thereby reversing the behavioural effects (anxiety, social aversion) associated with these suggesting a beneficial role against conditions associated with behavioural hyperactivity and social aversions as seen autistic condition. Indeed, previous studies have confirmed the involvement of adrenaline and 5-HT on anxiety and social aversion respectively (Chatterjee *et al*., 2015; Ben-Azu *et al*., 2017). The ability of Morin to reverse these VPA-induced neurotransmitter alteration in this study, suggests that it has some levels of neurotransmitter modulating activities as has been previously documented (Ben-Azu *et al*., 2017; Posar and Visconti, 2023).

The histomorphology of the CA1 region of the hippocampus was examined using haematoxylin and eosin (H&E) stain while neuronal cell bodies was examined via crystal fast violet (CFV) stain. Following exposure to VPA, the CA1 region showed several signs of neuronal degeneration, including sparse and irregularly arranged neural cells, with neuronal pyknosis. Similarly, Nissl stains revealed neuronal degenerative features like decrease in the number of Nissl bodies and increased chromatolytic cells in the CA1 region of the hippocampus of VPA exposed group. Previous studies have associated prenatal VPA exposure with neuronal degeneration, consistent with our findings (Petrenko *et al*., 2014; Wang *et al*., 2023). However, prenatal treatment with Morin significantly restored the cellular structure and protected the integrity of the neural cells in the offspring. This observation is in tandem with a past studies demonstrating bioflavonoid's ability to prevent neurodegeneration (Maher, 2019). Morin has been reported to possess free radical scavenging potential (Ogunlade *et al*., 2020; Bellavite, 2023), which confers on it the ability to protect the neuronal cells from cellular damage via oxidative stress.

Brain derived neurotrophic factor (BDNF) is one of the major neurotrophic factors that primarily support the development, regeneration, survival and maintenance of neuronal functions. It is synthesized by neurons in the striatum, frontal cortex and hippocampus of rodents. BDNF is the principal neurotrophin target of VPA in the foetal brain more so, supportive evidence has shown that neurological disorders are associated with alterations in neural growth factors in the nervous system (Zhong *et al*., 2020). In the

present study VPA decreased the number of BDNF-positive cells in the CA1 region of hippocampus, but Morin prenatal treatment increased these cells, promoting neurogenesis and mitigating certain autistic-like traits found in the behavioural tests carried out in this study. These traits include social skills and cognitive function, which have been linked to hippocampal neurogenesis in ASD (Klempin *et al*., 2013). Reports from several authors highlighted the BDNF promoting capacity of Morin (Ben-Azu *et al*., 2018; Martínez-Coria *et al*., 2024).The chemical pathologies induced by VPA, accounts for the perceived compromise in histomorphology, cellularity, Nissl profile and BDNF expression of the pyramidal cells of the hippocampus (Almeida *et al*., 2014). Agreeably, histopathological alterations in hippocampus will affect their proper anatomy and physiology.

Conclusion

In summary, this study revealed that in-utero Morin supplementation effectively mitigated the negative effects of VPA-induced neurodevelopmental damages in the offspring of epileptic Wistar rats. Seizure and/or its treatment have been shown from the results in this study to be detrimental to the developing foetus as it has severe neurodevelopmental consequences. These were evidenced in the memory impairment, anxiety, lack of sociability as well as neurotransmitters and oxido-infammatory dysregulation recorded in the study. The study demonstrated that Morin supplementation effectively improved anxiety problem, social behaviour, repetitive stereotyped behaviour and memory. The improvement in abnormal behaviours in the autistic offspring of epileptic Wistar rats following Morin supplementation may be associated with the fact that Morin supplementation altered monoamine neurotransmitter levels in hippocampal tissue, reduced oxidative stress and neuroinflammation in hippocampal tissue, and relieved neural cell damage by stimulating increased BDNF expression by the neural cells.

Acknowledgement

The authors are thankful to Dr Ben-Azu Benneth of the Department of Pharmacology, Faculty of Basic Medical Science, Delta State University, Abraka, for the provision of Morin used in this study.

Grants and Financial Support

This work was supported by the TETFUND Institution Based Research (IBR) grant.

Conflict of Interest

None declared.

Authors' Contribution

CA and OV- Conceptualized the idea, supervised the work and reviewed the final manuscript; OV and OI- Carried out the lab work, drafted the initial manuscript; OV- Carried out the statistical aspect of the work.

REFERENCES

Akinluyi, E., Aderibigbe, A., Olusegun, A., Ben-Azu, B., Eduviere, A., Ajayi, A., et al. (2020) Morin hydrate attenuates chronic stress-induced memory impairment and degeneration of hippocampal subfields in mice: The role of oxidative, nitrergic and neuroinflammatory pathways. Metab Brain Dis; 35(7):1145-1156.

Almeida, L.E., Roby, C.D. and Krueger, B.K. (2014) Increased BDNF expression in fetal brain in the valproic acid model of autism. Mol Cell Neurosci. 59:57–62. https://doi.org/10.1016/j.mcn.2014.01.007

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders: DSM-5™ (5th ed.). American Psychiatric Publishing, Inc. https://doi.org/10.1176/appi.books.9780890425596

Ayala, A., Muñoz, M.F., Argüelles, S. (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. O Oxid Med Cell Longev. 2014:360438.

Bauman, M.L. (2010) Medical comorbidities in autism: challenges to diagnosis and treatment. Neurotherapeutics; 7(3):320-327.

Bellavite, P. (2023) Neuroprotective potentials of flavonoids: experimental studies and mechanisms of action. Antioxidants (Basel). 12(2):280.

Ben-Azu, B., Aderibigbe, A.O., Ajayi, A.M., Eneni, A.O., Umukoro, S. and Iwalewa, E.O. (2018) Involvement of GABAergic, BDNF and Nox-2 mechanisms in the prevention and reversal of ketamine-induced schizophrenia-like behavior by morin in mice, Brain Res Bullet. 139:292-306,

Ben-Azu, B., Aderibigbe, A.O., Omogbiya, I.A., Ajayi, A.M. and Iwalewa, E.O. (2017) Morin pretreatment attenuates schizophrenia-like behaviors in experimental animal models. Drug Res. 68(03):159-167.

Ben-Azu, B., Emokpae, O., Ajayi, A.M., Jarikre, T.A., Orhode, V., Aderibigbe, A.O., et al. (2020) Repeated psychosocial stress causes glutamic acid decarboxylase isoform-67, oxidative-Nox-2 changes and neuroinflammation in mice: Prevention by treatment with a neuroactive flavonoid, morin. Brain Res. 1744:146917

Błaszczyk, B., Miziak, B., Pluta, R. and Czuczwar, S.J. (2022) Epilepsy in pregnancy-management principles and focus on valproate. Int J Mol Sci. 23(3):1369.

Cetin, F.H., Tunca, H., Güney, E.S.R.A. and Iseri, E. (2015) Neurotransmitter systems in autism spectrum disorder. Autism Spectrum Disorder—Recent Advances, 15-30

Cezar, L.C., Kirsten, T.B., da Fonseca, C.C.N., de Lima, A. P.N., Bernardi, M.M. and Felicio, L.F. (2018). Zinc as a therapy in a rat model of autism prenatally induced by valproic acid. Prog Neuro-Psychopharmacol and Biol Psychiatry. 84:173–180. doi:10.1016/j.pnpbp.2018.02.008

Chatterjee, M., Verma, R., Kumari, R., Singh, S., Verma, A.K., Dwivedi, A.K., et al. (2015) Antipsychotic activity of standardized Bacopa extract against ketamine-induced experimental psychosis in mice: Evidence for the involvement of dopaminergic, serotonergic, and cholinergic systems. Pharm Biol. 53:1850-1860.

Chen Y, Li Y, Xu H, Li G, Ma Y, Pang YJ (2017) Morin mitigates oxidative stress, apoptosis and inflammation in cerebral ischemic rats. Afr J Tradit Complement Altern Med; 13;14(2):348-355.

Chugani DC. (2011) Neurotransmitters. In: Amaral, D., Geschwind, D. and Dawson, G. (Eds) Autism Spectrum Disorders. New York; online edn, Oxford. https://doi.org/10.1093/med/9780195371826.003.0037

DiCarlo, G.E., Aguilar, J.I., Matthies, H.J., Harrison, F.E., Bundschuh, K.E., West, A., et al. (2019) Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. J Clin Investig. 129 (8):3407-3419. https://doi.org/10.1172/JCI127411

Edelstein, A.D., Mark, A.T., Nenad, A., Henry, P., Ronald, D.V., Nico, S. (2014). Advanced methods of microscope control using μManager software. J Biol Methods. 1: e10.

Fang, S.H., Hou, Y.C., Chang, W.C., Hsiu, S.L., Chao, P.D. and Chiang, B.L. (2003) Morin sulfates/glucuronides exert anti-inflammatory activity on activated macrophages and decreased the incidence of septic shock. Life Sci. 74:743–756

Galvez, J., Coelho, G., Crespo, M.E., Cruz, T., Rodriguez Cabezas, M.E., Concha, A., et al. (2001) Intestinal anti-inflammatory activity of Morin on chronic experimental colitis in the rat. Aliment. Pharmacol. Ther. 15:2027–2039.

Han, Y. and Chen, J.Z. (2013) Oxidative stress induces mitochondrial DNA damage and cytotoxicity through independent mechanisms in human cancer cells. BioMed Res Int. 2013:1-8. doi:10.1155/2013/825065.

Heydari, S., Mahmoudi, A., Amin, B., Pejhan, A., Mirmoosavi, S.J., Gholami, O., et al. (2021) Effects of camel milk on antioxidant activity in rats with valproic acid-induced autism. J Nutr Fast Health. 9(2):160-170. DOI: 10.22038/jnfh.2021.55516.1321.

Ishola, I.O., Balogun, A.O. and Adeyemi, O.O. (2020) Novel potential of metformin on valproic acid-induced autism spectrum disorder in rats: involvement of antioxidant defence system. Fundam Clin Pharmacol. 34(6):650-661.

Ishola, I.O., Olayemi, S.O., Yemitan, O.K. and Akinseye, K. (2015) Role for monoaminergic systems in the antidepressant and anxiolytic properties of the hydroethanolic leaf extract from Adeniacissampeloides. J Basic Clin Physiol Pharmacol. 26(3):301-312.

Johannessen, C.U. (2000) Mechanisms of action of valproate: a commentatory. Neurochem Int. 37:103–110.

Kapoor, R. and Kakkar, P. (2012) Protective role of Morin, a flavonoid, against high glucose induced oxidative stress mediated apoptosis in primary rat hepatocytes. PLoS One. 7:416–463.

Kawabata, K., Tanaka, T., Honjo, S., Kakumoto, M., Hara, A., Makita, H., et al. (1999) Chemopreventive effect of dietary flavonoid Morin on chemically induced rat tongue carcinogenesis. Int J Camcer. 83(3):381–386.

Klein, A. (2014) Epilepsy in women of childbearing age. In: Epilepsy. Miller, J.W. and Goodkin, H.P. (Eds.), 1st Edn. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118456989.ch25

Klempin, F., Beis, D., Mosienko, V., Kempermann, G., Bader, M. and Alenina, N. (2013) Serotonin is required for exercise-induced adult hippocampal neurogenesis. J Neurosci. 33(19):8270–8275. https://doi.org/10.1523/JNEUROSCI.5855-12.2013

Kraeuter, A.K., Guest, P.C. and Sarnyai, Z. (2019) The elevated plus maze test for measuring anxiety-like behavior in rodents. Methods Mol Biol. 1916:69–74. https://doi.org/10.1007/978-1-4939-8994-2_4

Kumar, H. and Sharma, B. (2016) Memantine ameliorates autistic behavior, biochemistry & blood brain barrier impairments in rats. Brain Res Bull. 124:27-39.

Laganà, A.S., Triolo, O., D'Amico, V., Cartella, S.M., Sofo, V., Salmeri, F.M., et al. (2016) Management of women with epilepsy: from preconception to post-partum. Arch Gynecol Obstet. 293(3):493-503.

Li, K., Li, L., Cui, B., Gai, Z., Li, Q., Wang, S., et al. (2018) Early postnatal exposure to airborne fine particulate matter induces autism-like phenotypes in male rats. Toxicol Sci. 162(1):189–199. https://doi.org/10.1093/toxsci/kfx240

Lin, W., Chan, Y.H., Kiing, J.S.H., Lim, T.S.H., Chong, S.C., Kang, Y.Q., et al. (2023) Restricted and repetitive behaviors and association with cognition and adaptive functioning in children with autism spectrum disorder in Singapore. Front Psychiatry 14:1249071.

Lyall, K., Schmidt, R.J. and Hertz-Picciotto, I. (2014) Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol. 43:443–464.

Maher, P. (2019) The potential of flavonoids for the treatment of neurodegenerative diseases. Int J Mol Sci. 20(12):3056.

Manivasagam, T., Arunadevi, S., Essa, M.M., SaravanaBabu, C., Borah, A., Thenmozhi, A.J., et al. (2020) Role of Oxidative Stress and Antioxidants in Autism. Adv Neurobiol, 24:193–206. https://doi.org/10.1007/978-3-030-30402-7_7

Martínez-Coria, H., Serrano-García, N., López-Valdés, H.E., López-Chávez, G.S., Rivera-Alvarez, J., Romero-Hernández, Á., et al. (2024) Morin improves learning and memory in healthy adult mice. Brain Behav. 14(2):e3444.

Matta, S.M., Hill-Yardin, E.L. and Crack, P.J. (2019) The inflfluence of neuroinflflammation in autism spectrum disorder. Brain Behav Immun. 79:75–90.

McPheeters, M.L., Warren, Z., Sathe, N., Bruzek, J.L., Krishnaswami, S., Jerome, R.N., et al. (2011) A systematic review of medical treatments for children with autism spectrum disorders. Pediatrics. 127(5):e1312–e1321.

Merali, Z., Presti-Torres, J., Mackay, J.C., Johnstone, J., Du, L., St-Jean, A., et al. (2014) Long-term behavioral effects of neonatal blockade of gastrin-releasing peptide receptors in rats: similarities to autism spectrum disorders. Behav Brain Res. 263:60–69.

Merwid-Ląd, A., Trocha, M., Chlebda, E., Sozański, T., Magdalan, J., Ksiądzyna, D., et al. (2012) Effects of Morin-5'-sulfonic acid sodium salt (NaMSA) on cyclophosphamide-induced changes in oxido-redox state in rat liver and kidney. Hum Exp Toxicol. 31(8):812–819.

Morgan, C.N., Roy, M. and Chance, P. (2003) Psychiatric comorbidity and medication use in autism: A community survey. Psychiatr Bull. 27(10):378–381.

Nagy, Z.B., Barták, B.K., Zsigrai, S., Kalmár, A., Szigeti, K., Igaz, P., et al. (2020). Effect of DNA fragments and nu-

cleotid supplementation on tumor development in mice model. Cancer Res. 80(S16):2761-2761.
NIH (1985) Guide for the Care and Use of Laboratory Animals. NIH Publication Number 85-23, US Department of Health, Education and Welfare. National Institutes of Health. Bethesda, MD.
Nijveldt, R.J., van Nood, E., van Hoorn, D.E., Boelens, P.G., van Norren, K. and van Leeuwen, P.A. (2001) Flavonoids: a review of probable mechanisms of action and potential applications, Am J Clin Nutr. 74:(4): 418–425. https: //doi.org/10.1093/ajcn/74.4.418
Nucera, B., Brigo, F., Trinka, E. and Kalss, G. (2022) Treatment and care of women with epilepsy before, during, and after pregnancy: a practical guide. Ther Adv Neurol Disord. 15:1-31.
Ogunlade, B., Afolayan, O.O. and Adelakun, S.A. (2020) Sulphoraphane supplementation ameliorates behavioural impairments and hippocampal neurodegeneration induced by lead exposure in adult Wistar rats. Nig J Neurosci. 11(2):88-98.
Pazvantoglu, O., Selek, S., Okay, I.T., Sengul, C., Karabekiroglu, K., Dilbaz, N., et al. (2009) Oxidative mechanisms in schizophrenia and their relationship with illness subtype and symptom profile. Psychiatry Clin Neurosci. 63: 693-700. https://doi.org/10.1111/j.1440-1819.2009.02015.x
Petrenko, A., Gulyaev, M., Tischenko, D., Petuchov, V. and Abbasova, K. (2014) Effects of prenatal treatment with valproic acid (VPA) on offspring of epileptic adult rats: mri investigation. Journal of Neuroscience and Neuroengineering. 2(6):1–7.
Ponou, B.K., Teponno, R.B., Ricciutelli, M., Quassinti, L., Bramucci, M., Lupidi, G., et al. (2010) Dimeric antioxidant and cytotoxic triterpenoid saponins from Terminalia ivorensis A. Chev. Phytochemistry. 71:2108–2115.
Posar, A. and Visconti, P. (2023) Autism spectrum disorder in 2023: A challenge still open. Turk Arch Pediatr. 58(6):566-571.
Posey, D.J., Erickson, C.A. and McDougle, C.J. (2008) Developing drugs for core social and communication impairment in autism. Child Adolesc Psychiatr Clin N Am. 17(4):787–801.
Qoronfleh, M.W. (2020) Role of oxidative stress and antioxidants in autism. Adv Neurobiol. 24:193-206.
Rein, B., Ma, K. and Yan, Z. (2020) A standardized social preference protocol for measuring social deficits in mouse models of autism. Nat Protoc. 15:3464–3477.
Roullet, F.I., Lai, J.K. and Foster, J.A. (2013) In utero exposure to valproic acid and autism--a current review of clinical and animal studies. Neurotoxicol Teratol. 36:47-56.
Rylaarsdam, L. and Guemez-Gamboa, A. (2019) Genetic causes and modifiers of autism spectrum disorder. Front Cell Neurosci. 13:385.
Sandhya, T., Sowjanya, J. and Veeresh, B (2012) Bacopa monniera (L.) Wettst ameliorates behavioral alterations and oxidative markers in sodium valproate induced autism in rats. Neurochem Res. 37:1121-1131.
Schneider, T. and Przewłocki, R. (2005) Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. Neuropsychopharmacology 30:80–89.
Schneider, T., Roman, A., Basta-Kaim, A., Kubera, M., Budziszewska, B., Schneider, K., et al. (2008) Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. Psychoneuroendocrinology. 33:728–740.
Schneider, T., Turczak, J. and Przewłocki, R.. (2006) Environmental enrichment reverses behavioral alterations in rats prenatally exposed to valproic acid: issues for a therapeutic approach in autism. Neuropsychopharmacology. 31(1):36-46.
Sreedharan, V., Venkatachalam, K.K. and Namasivayam, N. (2009) Effect of morin on tissue lipid peroxidation and antioxidant status In 1, 2- dimethylhydrazine induced experimental colon carcinogenesis. Invest New Drugs. 27:21 –30
Subash, S. and Subramanian, P. (2009) Morin a flavonoid exerts antioxidant potential in chronic hyperammonemic rats: a biochemical and histopathological study. Mol Cell Biochem. 327:153–161.
Tomson, T. (2005) Gender aspects of pharmacokinetics of new and old AEDs: pregnancy and breastfeeding. Ther Drug Monit. 27:718–772.
Trovato Salinaro, A., Pennisi, M., Di Paola, R., Scuto, M., Crupi, R., Cambria, M.T., et al. (2018) Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: modulation by nutritional mushrooms. Immun Ageing. 15:1-8.
Usui, N., Kobayashi, H. and Shimada, S. (2023) Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. Int Mol Sci. 24(6):5487. https://doi.org/10.3390/ijms24065487
Veiby, G., Engelsen, B.A. and Gilhus, N.E. (2013) Early child development and exposure to antiepileptic drugs prenatally and through breastfeeding: a prospective cohort study on children of women with epilepsy. JAMA Neurol. 70:1367–1374.
Veroniki, A.A., Cogo, E., Rios, P., Straus, S.E., Finkelstein, Y., Kealey, R., et al. (2017) Comparative safety of antiepileptic drugs during pregnancy: a systematic review and network meta-analysis of congenital malformations and prenatal outcomes. BMC Med. 15:95.
Vlasov, P. (2023) Perspective Chapter: Epilepsy and Pregnancy. Epilepsy During the Lifespan - Beyond the Diagnosis and New Perspectives. IntechOpen.. http://dx.doi.org/10.5772/intechopen.111957
Wagner, G.C., Reuhl, K.R., Cheh, M., McRae, P. and Halladay, A.K. (2006) A new neurobehavioral model of autism in mice: pre- and postnatal exposure to sodium valproate. J Autism Dev Disord. 36(6):779–793. https://doi.org/10.1007/s10803-006-0117-y
Wang, L., Almeida, L.E., Spornick, N.A., Kenyon, N., Kamimura, S., Khaibullina, A., et al. (2015) Modulation of social deficits and repetitive behaviors in a mouse model of autism: the role of the nicotinic cholinergic system. Psychopharmacology. 232(23):4303-4316.
Wang, L., Wang, B., Wu, C., Wang, J. and Sun, M. (2023) Autism spectrum disorder: Neurodevelopmental risk factors, biological mechanism, and precision therapy. Int J Mol Sci. 24:1819.

Wang, Y., Zhang, H. and Yuan, Q. (2013) Purification, antioxidant activity and protein-precipitating capacity of punicalin from pomegranate husk. Food Chem. 138:437–443.
Wing, L., Gould, J. and Gillberg, C. (2011) Autism spectrum disorders in the DSM-V: better or worse than the DSM-IV?. Res Dev Disabil. 32(2):768–773.
Wu, H., Zhao, G., Liu, S., Zhang, Q., Wang, P., Cao, Y., et al. (2022) Supplementation with selenium attenuates autism-like behaviors and improves oxidative stress, inflflammation and related gene expression in an autism disease model. J Nutr Biochem. 107:109034.
Zhang, Y., Sun, Y., Wang, F., Wang, Z., Peng, Y. and Li, R. (2012) Downregulating the canonical Wnt/β- catenin signaling pathway attenuates the susceptibility to autism-like phenotypes by decreasing oxidative stress. Neurochem Res. 37(7):1409-1419.
Zhong, H., Xiao, R., Ruan, R., Liu, H., Li, X., Cai, Y., et al. (2020). Neonatal curcumin treatment restores hippocampal neurogenesis and improves autism-related behaviors in a mouse model of autism. Psychopharmacology. 237:3539–3552. doi: 10.1007/s00213-020-05634-5.

Published by Neuroscience Society of Nigeria