ORIGINAL ARTICLE

Official Journal of the Neuroscience Society of Nigeria (NSN)

http://doi.org/10.47081/njn2020.11.1/002
ISSN 1116-4182

Prenatal Supplementation with *Curcuma longa* Ameliorates Oxidative Stress, Improves Behaviour and Hippocampal Alterations in Valproic Acid-Induced Autism in Sprague-Dawley Rat

Stella C. Gbotolorun[1], Comfort O. A. Okoh[1], Yewande O. Oshodi[2]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Nigeria
[2]Department of Psychiatry, Faculty of Clinical Sciences, College of Medicine, University of Lagos, Idi-Araba, Nigeria

Received: ……. March 2020
Accepted: ……. May 2020

ABSTRACT

There is growing global number of persons affected with autism, and teratogenic influences arising from several epigenetic factors have been implicated. This study aimed to determine the effect of prenatal supplementation of extract of *Curcuma longa* on valproic acid-induced model of autism in Sprague-Dawley rats. Thirty pregnant Sprague-Dawley rats divided into five groups (n=6) were used. Valproic acid (500 mg/kg body weight) induced autism on gestation day 12.5. The groups were designated as control, valproic acid, and valproic acid with 5, 10 and 20 mg/kg body weights *Curcuma longa* respectively, (*Curcuma longa* was administered from day 1-21 of gestation). On postnatal day 21, five male pups were randomly selected from each group, and neurobehavioral tests were performed until postnatal day 28. The pups were sacrificed on postnatal day 28, and the hippocampus was dissected for histology and biochemical assays. Treated groups showed improvement in anxiety and social behaviour. The histological sections showed fewer atrophied cells, reduced degree of chromatolysis with better delineation of the cells within the pyramidal layer compared with valproic acid group. Dopamine, lL-6 and TGF β1 levels were not significantly different from control. Malondialdehyde and glutathione values of the treated groups were significantly different from valproic acid groups. Superoxide dismutase and catalase showed no significant difference when treated groups were compared to valproic acid group except the medium dose for catalase. This study shows that prenatal supplementation with *Curcuma longa* is a potential ameliorative agent against teratogenic epigenetic agents that may lead to autism.

Key words: *Curcuma longa, Valproic acid, Autism, Anxiety, Epigenetic, Social behaviour*

INTRODUCTION

Autism is an idiopathic developmental disorder that is majorly characterized by deficits in social interaction and communication, alongside stereotyped behaviours with restricted interests (Won et al. 2013). There is a global rise in the prevalence of the disorder, being common in males than females in a 3:1 ratio (Loomes et al. 2017). In Nigeria, Autism has been shown to occur at a rate of 11.4% among children with developmental disabilities (Bakare et al. 2012). Autistic features have been successfully mimicked with the generation of several rodent models. Valproic acid-induced model of autism is one of the non-genetic models of autism, used to investigate the neurobiology underlying autistic behaviour (Nicolini and Fahnestock 2018; Patterson

Correspondence: Stella C. Gbotolorun, PhD, Department of Anatomy, Faculty of Basic Medical Sciences, University of Lagos, Idi-Araba, PMB 12003, Lagos, Nigeria. Email: sgbotolorun@unilag.edu.ng; Phone: +2348038098631

2011). Phenotypic features seen in rodents that have been induced with autism are in the form of shortened and crooked tails (Choi et al. 2016).

In humans, variation occurs in the area of the brain affected in autism, this may be due to the extent of severity of the disorder in an individual (Courchesne et al. 2007). Some studies have indicated involvement of the *Cornu Ammonis* 3 (CA3) subfield of the hippocampal pyramidal cells in the neuropathology of autism (Courchesne et al. 2007; Li et al. 2019). Neuronal malformations within this region of the hippocampus may be associated with deficits in memory processing and speech. The subsequent disruption in communication in autistic spectrum disorder (ASD) individuals especially in children may imply an inability to effectively express through language the spatial perception of the environment (Landau 2017).

The debilitating financial implication on the nation coupled with the physical, psychological and emotional trauma the families of affected individuals would have to contend with heightens the importance for appropriate intervention (Bakare et al. 2012). Nigeria still lags behind in the knowledge about the management of autism due to several factors among which are cultural beliefs and practices, inadequate number of trained personnel, inadequate facilities and inadequate and tortuous health care system (Bakare et al. 2009). ASD represents a significant public health problem and a huge burden for education and social service systems (Franz et al. 2017). The present treatment strategies, majorly antipsychotics (risperidone and aripiprazole), have been proven effective in the management of the core symptoms of autism. However, these interventions can cause the generation of debilitating side effects after long term use (LeClerc and Easley 2015). Present investigations into the use of prenatal vitamins/irons as recommended by the World Health Organisation for women of child-bearing age raises cause for concern (WHO 2012). This is because studies have suggested that there may be some epigenetic teratogenic implications on the foetuses that may lead to the advent of autism depending on the concentration of the drugs (van Gelder et al. 2011; Wiens and DeSoto 2017). Researchers have discovered that nutraceuticals have complementary properties that could help reduce the degenerative and inflammatory adverse effects induced after long term use of pharmacotherapeutics. They have also reported that these nutraceuticals help in the management of core symptoms associated with the disorder (Bang et al. 2017). One of such nutraceuticals is curcumin; the most active phenolic component of the rhizome turmeric (*Curcuma longa*). It constitutes about 2-5% of the spices and gives it the characteristic yellow-orange colouration (Bagchi 2012). Commercially, it is used as a natural colouring agent for food, cosmetics and dye and as an active ingredient in some medicines (Karim et al. 2010). The medical benefits of curcumin cannot be overemphasized. It has been proven to be beneficial in alleviating health challenges associated with numerous diseases due to its excellent anti-inflammatory and antioxidant properties (Cox et al. 2015).

Some authors have emphasized on the importance of early detection of children living with ASD suggesting that this would reduce the occurrence and subsequent stigma that may occur later in life (Oshodi et al. 2016). However, others have reported that early detection alone may not suffice. These authors recommended that pre-treatment with curcumin may have more ameliorative effect against some of the biomarkers associated with autism (Al-Askar et al. 2017).

Therefore, this study was carried out to investigate the effect of prenatal administration of ethanolic extract of *Curcuma longa* (*C. longa*) against the inflammatory responses and behavioural alterations associated with autism. Ethanolic extract of turmeric has been proven to contain a high yield of curcumin (Liu et al. 2008). It further attempts to elucidate a mechanism by which the extract exerts the proposed ameliorative effect in relation to the hippocampal role in spatial orientation and memory.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee; College of Medicine of the University of Lagos with approval number- CMUL/HREC/02/19/494.

Animal Handling

A total of thirty healthy cyclic female Sprague-Dawley rats (100-120 g), about 7-8 weeks old were procured from the animal house of the College of Medicine, University of Lagos, Nigeria. The animals were housed in cages, and were left to acclimatize for two weeks before the commencement of the study. The animals had free access to chow pellets and water ad libitum. They were maintained on a 12-12h light-dark cycle, at a controlled temperature of 25±2℃ and relative humidity of 50-70%.

Valproic Acid (VPA) Induced Model of Autism

Sodium valproate (valproic acid) was purchased from Emzor Pharmacy and Stores, Lagos, Nigeria, as 500 mg tablets; and batch number- 6A584, 566452. Each 500 mg tablet was dissolved in 10 mL of distilled water at a concentration of 50 mg/ml. Pregnant dams received a single intraperitoneal injection of 500 mg/kg on gestation day 12.5 (Kataoka et al. 2013).

Extraction and Administration of Curcumin

Fresh turmeric rhizomes weighing 2000 g was obtained from mile 12, a local market in Lagos State,

Nigeria. They were taken to the herbarium of the Botany Department, University of Lagos, for authentication. Catalogue serial number, 8008 was assigned to it. Extraction was done adopting methodologies from previous work with slight modifications (Bagchi 2012). The rhizomes were washed with clean water then chopped into sizable pieces of about 1 cm^3 with a grater, then placed in a pulverised thermostatic oven at 40℃ for 72 h. The dried sample was grinded into powder form with an electric grinder. The dried weight obtained (300.14 g) was extracted with 80% ethanol solution in a Soxhlet apparatus for 24 h. The extract was concentrated with a rotary evaporator and later dried over a water bath at 40℃ for 48 h. A semisolid extract weighing 67 g was obtained. Three different doses of the extract (5, 10, and 20 mg/kg) were chosen based on the modifications of a previous study by Xu et al. (2007). The extract was administered orally using a cannula.

Determination of Pregnancy

Vaginal smear was taken daily, and vaginal cytology was performed to monitor the oestrous cycle. Females on the late proestrous phase were placed in separate cages with male animals of proven fertility in a ratio 2:1 for the purpose of mating (Gbotolorun et al. 2012). Pregnancy was established when spermatozoa was observed in the vagina smear in the morning after mating and was counted as day 0.5 of gestation.

Experimental Grouping

The pregnant rats were randomly divided into 5 groups of 6 animals each immediately pregnancy was confirmed, and treatment commenced. The groups were designated as control (received 1 mL distilled water), VPA (500 mg/kg), and VPA with 5, 10 and 20 mg/kg body weight of *C. longa* respectively. The pregnant dams were housed individually in cages and were monitored throughout the period of pregnancy. They were allowed to litter and nurse their pups until weaning at postnatal day (PND) 21 (Kataoka et al. 2013). Five male pups were then randomly selected from each group and were used for the experiment.

Neurobehavioral Studies

Behavioural tests were carried out from PND 21 to PND 28. Immediately after each trial, cotton pad was used to pick up faeces, and then a towel soaked with 70% ethanol was used to clean up the entire apparatus. They were then left to air dry before a new test subject rat was introduced. A Tony camera video device was also used alongside manual recordings.

Table 1: The effect of ethanolic extract of *C. longa* on the number of arm entries and time spent in either arm of the elevated plus maze in VPA model of autism

Groups	Open Arm	Closed Arm	Open Arm Time (sec)	Closed Arm Time (sec)
Control	1.20±0.58	3.20±1.16	75.36±57.42	224.60±57.42[d,e]
VPA	2.00±1.00	3.00±0.58	22.00±11.01	278.00±11.01[b,d,e]
5 mg/kg	0.67±0.33	1.00±0.58	128.80±89.16	171.20±89.16
10 mg/kg	1.60±0.87	5.40±0.75[a,c,ci,d]	21.36±13.51	278.80±13.39[d,a,b,e]
20 mg/kg	1.80±0.58	2.20±0.49	26.64±10.12	273.40±10.12[e,a,b,d]

Values are expressed as mean ± Standard error of mean (SEM); (N=5). a =p<0.05 significant compared to control group in the open arm; b =p<0.05 significant compared to VPA group in the open arm; c and ci =p<0.05 significant compared to 5 mg/kg body weight group in the open and closed arm respectively; d =p<0.05 significant compared to the open arm of 10 mg/kg body weight group; e =p<0.05 significant compared to 20 mg/kg body weight group in open arm

Crawley's Sociability and Preference for Social Novelty Test

A three-chambered apparatus was used for this test. It consisted of a rectangular box made of Plexiglas

Table 2: The effect of ethanolic extract of *C. longa* on the number of entries and time spent by subject rat in chambers containing empty cusp, stranger 1, and stranger 2 rats in VPA model of autism

Groups	Stranger 1	Empty	Stranger 1	Stranger 2	Stranger 1 (sec)	Empty (sec)	Stranger 1	Stranger 2
Control	3.60±1.03	1.60±0.81[b]	1.40±0.93	0.40±0.40	369.10±104.90	19.08±11.19[a]	358.80 ±112.20	11.92 ±7.40[a]
VPA	6.00±0.00	5.00±0.58	3.00±0.58	3.33±0.88	342.80±18.29	129.40±21.62[b]	227.40 ±66.04	299.60 ±38.64
5 mg	3.67±0.33	2.33±0.88	4.67±3.28	5.33±3.93	419.60±86.70	68.80±32.09[c]	309.20 ±150.50	248.40 ±130.00
10 mg	4.00±1.30	1.80±0.58	2.20±0.86	2.80±1.07	398.80±104.00	159.80±108.10	215.60 ±101.00	283.20 ±99.51
20 mg	2.80±0.86	1.00±0.63[b]	2.80±0.58	1.80±0.74	324.50±102.20	43.62±27.32[e]	271.90 ±46.36	221.80 ±67.49

Values are expressed as mean ± Standard error of mean (SEM); (n=5). a =p<0.05 significant compared to control group in stranger 1 rat; b =p<0.05 significant compared to VPA group in stranger 1 rat; bi =p<0.05 significant compared to VPA group in stranger 1 rat; c =p<0.05 significant compared to 5 mg/kg body weight group in stranger 1 rat; e =p<0.05 significant compared to 20 mg/kg body weight group in stranger 1 rat

(405 mm wide x 600 mm long x 150 mm high) divided into three-identical-chambers (405 mm wide x 200 mm long x 150 mm high) by two Plexiglas walls containing small openings which measure 100 mm wide x 50 mm high, to allow mouse access between chambers as described by (Kaidanovich-Beilin et al. 2011). The test involved the free choice of the rat to either initiate or disengage interaction with one or two unfamiliar, stranger rats. It comprised of two experimental sessions after five minutes of acclimatization within the enclosed middle chamber.

Session one started as an unfamiliar rat, stranger 1 is put into one of the wired cups located within the other two chambers for ten minutes. Within this time, number of entries into each chamber with containment cups either housing or not housing stranger 1 and the middle chamber was counted. Also, duration of stay in each chamber and number of active contacts with the containment cups were noted.

Then session two began for another ten minutes as stranger 2 is introduced into the empty containment cup. This is the social novelty or preference session. Similar parameters were noticed as in session one but in this case, behavioural responses of subject rat to the presence of stranger 2 were noted (Kaidanovich-Beilin et al. 2011).

Table 3: Effect of ethanolic extract of *C. longa* on total number of active paw contact and cusp-climbing into chambers containing stranger 1 and 2 rats in VPA model of autism

Groups	Total number of active paw contact		Total number of cusp-climbing	
	Stranger 1	Stranger 2	Stranger 1	Stranger 2
Control	15.20 ± 5.38[ai]	1.20 ± 0.97	0.40 ± 0.40	0.20 ± 0.20
VPA	16.00 ± 6.08	16.00 ± 1.00	0.33 ± 0.33	0.67 ± 0.67
5 mg/kg	14.33 ± 3.28	8.00 ± 4.00	2.33 ± 1.86	3.67 ± 2.03
10 mg/kg	11.00 ± 3.26	4.40 ± 1.21	4.80 ± 1.46	2.00 ± 1.05
20 mg/kg	6.80 ± 1.91	4.60 ± 2.75	1.00 ± 0.45	1.00 ± 0.45

Values are expressed as mean ± Standard error of mean (SEM); (n=5). ai =p<0.05 showed significant increase in paw contact with stranger 1 cusps in control group.

Elevated Plus Maze

This behavioural apparatus was used to test for anxiety related behaviours in test subjects. It consisted of two open and two closed arms. Rat elevated plus maze is approximately 50 cm long, 10 cm wide, with the closed arms having 30 cm high walls. The arms of the maze are attached to sturdy legs making it elevated at least 50 cm from the ground. Each test subject was placed on the maze in such a manner that it faces one of the open arms. Duration for the experimentation was about five minutes. Parameters considered were the number of times transition was made into each of the arms and the duration of stay (Tejada et al. 2009).

Animal Sacrifice and Perfusion

Male pups were sacrificed on PND 28 after being anaesthetized with 0.3 mL/kg of ketamine hydrochloride. The whole body was perfused transcardially with normal saline (for the purpose of biochemical assays) then followed by 10% buffered formal saline (for the purpose of haematoxylin and eosin (H&E) and Nissl light microscopic staining). The brain tissues for biochemical assays were cryoprotected by immersion in 30% sucrose at 4℃. The brain tissues were sectioned in the coronal plane to isolate the hippocampal formation in the laboratory for further investigations.

Tissue Preparation

Brain tissue (0.5 g) was homogenized using tissue homogenizer (Teflon machine) at 2,000 rpm, in 2 mL of 0.25 M sucrose buffer (pH 7.0). It was then centrifuged at 3,500 rpm for 10 min and the supernatant removed for the assay.

Determination of Reduced Glutathione (GSH) Activity

Total GSH was determined by the method of Tietze's enzymatic recycling assay with modifications (Rukkumani et al. 2004). The serum was incubated for 25 min at 37℃ in a medium containing 0.24 mM nicotinamide adenine dinucleotide phosphate (NADPH), 6.3 mM ethylenediamine tetra acetic acid (EDTA), 0.67 mM 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) and 143 mM sodium phosphate pH 7.5. After the addition of 1U/mL glutathione reductase (GRd), the absorbance increases at 412 nm due to the formation of 5-thio-nitrobenzoate was recorded. The concentration values were calculated from a GSH curve.

Determination of Lipid Peroxidation Activity

Rat malondialdehyde (MDA) enzyme-linked immunoassay (ELISA) kit was used to quantify the amount of lipid peroxidation **(**LPO) in the sample. The assay was done by the method described by Wright and colleagues with some modifications (Wright et al. 2003). The reaction mixture in a total volume of 3 mL contained 1 mL tissue homogenate, 1 mL of TCA (10%), and 1 mL TBA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were shifted to ice bath and then centrifuged at 2500×g for 10 min. The amount of MDA formed in each of the samples was assessed

by measuring the optical density of the supernatant at 532 nm.

Determination of Catalase (CAT) Activity

CAT (catalogue number- MBS701713 Elisa Kit) was assayed calorimetrically at 620 nm and expressed as moles of hydrogen peroxide (H_2O_2) consumed/min/mg protein as described (Quinlan et al. 1994). The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M with pH 7.0 sucrose buffer, 0.1 mL of plasma and 0.4 mL of 2M H_2O_2. The reaction was stopped by the addition of 2 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Determination of Superoxide Dismutase (SOD) Activity

Total SOD activity in tissue homogenates was determined following the procedure of Marklund and Marklund (1974) with slight modifications. The method is based on the activity of SOD to inhibit the autoxidation of pyrogallol. In 970 µL of buffer (100mM Tris-hydrocloric acid (Tris-HCl), 1 Mm EDTA, pH 8.2), 10 µL of homogenates and 20 µL pyrogallol (13 Mm) were mixed. Assay was performed in thermostated cuvettes at 25℃ and changes of absorption were recorded by a spectrophotometer at 480 nm. One unit of SOD activity was defined as the amount of enzyme that can inhibit the autoxidation of 50% the total pyrogallol in the reaction.

Determination of Neurotransmitter- Dopamine

The dopamine D_2 receptor (D_2R) ELISA kit was used according to manufacturer instructions. Briefly, 50 µL of the prepared standard was added to 10 µL then 40 µL of sample diluents into sample wells. Then 100 µL of horseradish peroxidase (HRP)-conjugate was further added then covered with an adhesive strip for 60 min at 37℃. Sample well was aspirated and washed. The optical density was read at 450 nm using a microtiter plate reader within 15 min. The standard curve was used to determine the amount of dopamine in the sample. The sensitivity by this assay was 10 pg/mL.

Determination of Pro-inflammatory Cytokine (IL-6)

This was carried out using ELISA as indicated by the manufacturer's protocol. At room temperature, all materials and prepared reagents were equilibrated. 100 µL of each standard and sample solution were added to each wells. Wells were covered and incubated for 2.5 h at room temperature. After subsequent washing the 100 µl of 1× biotinylated IL-6 detection antibody for 1 h, the solution was discarded then 100 µL of 1X HRP-streptavidin solution was added for 45 min after which 100 µL of TMB One-step substrate reagent was put into each well. This was incubated for 30 min. Then 50 µL of stop solution was added to each well and read at 450 nm immediately.

Determination of Anti-inflammatory Cytokine (TGF-β1)

The transforming growth factor β1 ELISA kit was used. First, 50 µL was added to the standard well. Then 10 µL testing sample and 10 µL sample diluents were also added to the sample well. Thereafter, 100 µL of HRP-conjugate reagent was added to each well and covered with an adhesive strip, then left to incubate for 60 min at 37℃. The solution was aspirated and washed with 400 µL wash solution. Chromogen A and B (50 µL each) were added for 15 min. Stop solution was put into the solution then optical density was read at 450 nm using a microtiter plate reader within 15 min. A standard curve was used to determine the amount of TGF-β1 in each sample.

Table 4: The effect of ethanolic extract of *C. longa* on oxidative stress markers in VPA-induced autism

Groups	SOD (U/mg/protein)	GSH (U/mg/protein)	MDA (U/mg/protein)	CAT (U/mg/protein)
Control	16.71 ± 1.05	18.54 ± 1.63	0.34 ± 0.01	16.89 ± 2.35
VPA	12.89 ± 0.00	30.10 ± 0.00[a]	1.05 ± 0.00[a]	8.34 ± 0.00
5 mg/kg	13.04 ± 4.44	15.62 ± 0.09[b]	0.33 ± 0.01[b]	16.89 ± 3.37
10 mg/kg	17.85 ± 9.01	15.94 ± 0.14[b]	0.32 ± 0.01[b]	34.90 ± 4.61[b]
20 mg/kg	9.00 ± 0.01	19.45 ± 0.26[b]	1.05 ± 0.02[a,c,d]	14.62 ± 2.55

Values are expressed as mean ± Standard error of mean (SEM); (n=5). a = p<0.05 significant compared to control group; b = p)0.05 significant compared to VPA group; c = p<0.05 is significant compared to 5 mg/kg body weight group; d = p<0.05 compared to 10 mg/kg body weight group;

H & E staining for light microscopy

Transverse sections of each hippocampus was made and fixed in 10% buffered formaldehyde solution. The tissues were dehydrated in ascending grades of alcohol and embedded in paraffin wax at 60℃. Tissue sections were made at 5 µm and slides were stained with routine haematoxylin and eosin.

Nissl Staining

Cresyl violet was used for counterstaining the light microscopic sections. The procedure followed that of the conventional light microscopic staining but with some modifications (Paul et al. 2008). Reagents included; 95% ethanol 70% ethanol differentiation solution: 2 drops glacial acetic acid in 95% ethanol, cresyl violet acetate 0.2% in acetate buffer. Initially, tissue sections were 'defatted' by passing through graded dilutions of ethanol (15 min in 100% xylene [3

changes], then 10 min in 100% ethanol). Xylene was used for dewaxing. They were then rehydrated by passing back through decreasing concentrations of ethanol then back into water. The ethanol solutions acted to differentiate the stain, causing myelin and other components to lose colour whereas the cell body (cytoplasm) retained the colour. Photomicrographs of all histological processes were made at x400 magnification using Olympus and Leica microscopes.

Statistical Analysis

Statistical analysis was performed for descriptive statistics and represented as mean ± standard deviation using Graphpad prism version 7.03 (GraphPad Inc., USA). Student t-test was used for comparison within groups; one-way analysis of variance was used for comparison between groups followed by Tukey's multiple comparisons test. The results were expressed as mean ± standard error of mean (SEM) and statistical significance was set at $p<0.05$.

RESULTS

Elevated Plus Maze:

Total Number of Arm Entries

There was no significant difference in total number of entries into both the open and closed arms across all groups when compared to control, except the 10 mg/kg body weight group, which showed a significant decrease in entries into the open arms. Total number of entries into the closed arm of 10 mg/kg body weight group showed significant increase when compared to the open arms of control group. Also, the 10 mg/kg body weight group showed significant increase in closed arm entry, when compared to both the open and closed arms of 5 mg/kg body weight group. Both groups 5 and 10 mg/kg body weight had the highest and least number of entries respectively, into either arm of the maze (Table 1).

Total Time Spent in Either Arms of the Maze

Multiple comparisons showed no significant difference in total time in both the closed and open arms alone across all groups. However, there was significant decrease in the open arm time when compared with the closed arm time in the VPA, 10 and 20 mg/kg body weight groups.

Across the groups, the open arm time of control group showed significant decrease when compared with the closed arms of groups 10 and 20 mg/kg body weights. Furthermore, the VPA group open arm time also showed significant decrease when compared with groups 10 and 20 mg/kg body weights closed arm time. The open arm of group D showed significant decrease in time when compared to the closed arm of group E and vice versa. In summary, 5 mg/kg body weight group spent less time while 10 mg group spent the greater time in exploring the arms of the maze (Table 1).

Crawley's Sociability and Social Novelty Test

Entries into the Chamber with the First Unfamiliar Rat or Empty Cusp

There was no significant difference in the total number of entries into the chamber containing the first unfamiliar rat (stranger 1) and that containing the empty cusp, across all the groups. However, the study recorded a significant increase in the total number of subject entries into the chamber containing stranger 1 in VPA group, when compared with the total number of entries into the empty cusp chamber in both control and 20 mg/kg body weight groups respectively. VPA group recorded lesser number of entries compared to 10 mg/kg body weight group which had the greatest number of entries into either chambers (Table 2).

Table 5: Effect of ethanolic extract of *C. longa* on inflammatory cytokines and dopamine levels in VPA-induced autism

Groups	IL-6 (ng/ml)	TGF β1 (ng/ml)	Dopamine (ng/ml)
Control	63.67±2.99	17.43±0.13	212.70±11.2
VPA	86.09±0.00	22.04±0.00	208.00±0.00
5 mg/kg	67.00±1.03	16.49±0.22	173.50±50.38
10 mg/kg	83.72±0.52	15.46±0.09	230±105.70
20 mg/kg	80.45±12.75	7.57±2.68[a,b,c]	236.10±1.50

Values are expressed as mean ± Standard error of mean (SEM); (N=5). "a =p<0.05 significant increase in TGF β1 in control group compared to 20 mg/kg body weight group; b =p<0.05 statistically significant increase in TGF β1 in VPA group compared to 20 mg/kg body weight group; c =p<0.05 significant increase in TGF β1 in the 5 mg/kg body weight group compared to 20 mg/kg body weight group;.

Entries into the Chambers with the First or Second Unfamiliar Rats

There was no significant difference in the total number of entries into the chamber containing the first unfamiliar rat (stranger 1) when compared to the entries into the chamber containing the second unfamiliar rat (stranger 2) across all groups. While control and 20 mg/kg body weight groups showed the least number of entries into either chamber, 5 mg group showed the highest number of entries (Table 2).

Time Spent in the Chambers with the First Unfamiliar Rat or Empty Cusp

All groups showed significant increase in total time spent in the chamber with the first unfamiliar rat

(stranger 1), than the chamber containing the empty cusps except 10 mg/kg body weight group. However, VPA group spent the least time exploring either chamber unlike the control group which spent the highest time (Table 2).

Time Spent in the Chambers with the First and Second Unfamiliar Rats

There was no significant difference in the total time spent in the chamber with stranger 1 rat in comparison with the chamber containing the stranger 2 rat across all groups, except in the control group which showed a significant increase in the time spent in the chamber with the stranger 1 rat. Control group showed the least time while VPA group the most time in either chamber (Table 2).

Active Paw Contact of Test Subject with Cusps Restricting Stranger Rats

There was no significant difference in active paw contact of subject rat to both stranger 1 and stranger 2 across all groups except control group, which showed an increased number of contacts to stranger 1. VPA group showed equal active contacts between cusps in both chambers while control group showed the least contact (Table 2).

Climbing of Cusps by Subject Rats

No significant difference occurred in the total number of cusps climbing across all the groups. However, the 5 mg/kg body weight group had the highest number of climbs while the 10 mg/kg body weight group had the least number. Meanwhile the 20 mg/kg body weight group rats had equal number of climbs in both stranger 1 and 2 chambers (Table 3).

Oxidative Stress Markers

No significant difference was observed in SOD activities across all treatment groups. GSH activity showed a significant increase in VPA group when compared to control group. No significant difference was observed when groups 5, 10 and 20 mg/kg body weights were compared to the control. However, they recorded significant reductions when compared to VPA group. MDA activities recorded a significant increase in VPA and 20 mg/kg body weight groups when compared to control group. But groups 5 and 10 mg/kg body weights recorded a significant decrease when compared to VPA and 20 mg/kg body weight groups respectively. CAT did not show any significant difference when the treatment groups were compared with the control. However, a significant increase was observed when the 10 mg/kg body weight group was compared with VPA group (Table 4).

Inflammatory Cytokines and Dopamine

There were significant increases in the pro-inflammatory cytokines (IL-6) when compared to the anti-inflammatory cytokines (TGF β1) across all

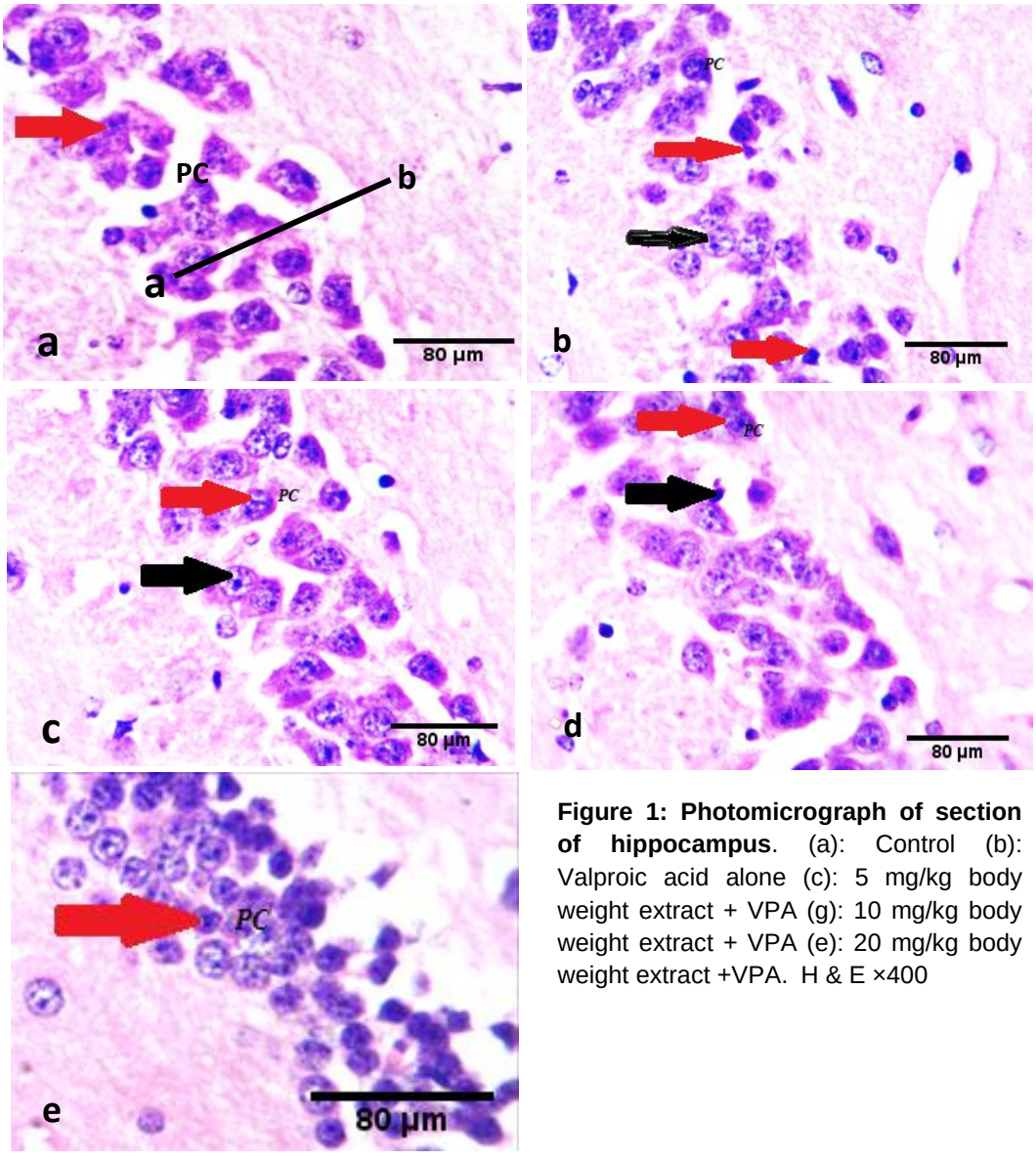

Figure 1: Photomicrograph of section of hippocampus. (a): Control (b): Valproic acid alone (c): 5 mg/kg body weight extract + VPA (g): 10 mg/kg body weight extract + VPA (e): 20 mg/kg body weight extract +VPA. H & E ×400

groups. There were no significant differences in the dopamine levels across all groups (Table 5).

Histoarchitecture of CA3 Pyramidal Neuronal Cells

Figure 1 shows photomicrographs of H&E staining of CA3 pyramidal neuronal cells. The control group showed pyramidal cells and proper cell delineation within the pyramidal layer, formed by densely packed rounded neurons. Cell cytoplasm appeared to be well preserved (Fig. 1a). There was poor delineation (scattering pattern) of the pyramidal cells within the pyramidal layer of the CA3 sub-region in the VPA group (Fig. 1b) compared to the control group. However, there was improvement in treatment groups 5, 10 and 20 mg/kg body weight; the pyramidal cell structure appeared better delineated from the surrounding layers and also presented a better stained cytoplasm (Figs. 1c-1e) than that of the VPA group. These positive features were more prominent in 5 mg/kg body weight treatment group compared to the others.

Figure 2 shows photomicrographs of special stain to demonstrate the Nissl profiling of the CA3 sub-region. Control rat demonstrated deeply stained, properly aligned pyramidal cells in the pyramidal layer (Fig. 2a). There was chromatolysis in the VPA group (Fig. 2b) compared to control. This chromatolytic appearance appeared to be less prominent across the *C. longa* treated groups (Fig. 2c-2e).

DISCUSSION

The rise in the prevalence of autism around the world has alerted researchers towards identifying several contributing factors *vis-à-vis* environmental and genetic factors that may possibly be responsible for this disorder. There is an increasing rise in the use of nutraceuticals in the treatment of sicknesses and diseases globally and this rise has been attributed to the perceived belief that nutraceuticals generate fewer side effects on long term use when compared to pharmacotherapeutics (Bang et al. 2017).

A number of unforeseen epigenetic factors pose a threat to the developing foetus. Valproic acid is used in the management of psychotic disorders in humans but when administered during the period of neural tube closure in rodents, it manifests core autistic features in the pups (Kataoka et al. 2013).

In this present study, ethanolic extract of *C. longa,* was administered prenatally throughout pregnancy to ascertain its role as a possible ameliorative or preventive agent in the autistic disorder. The involvement of the hippocampus alone cannot account for all the core symptoms associated with autism, however, abnormalities in the hippocampal pyramidal cells have been noted in the autistic disorder (Takano 2015). H&E stained sections of the present study showed a poor delineation (scattering pattern) of the pyramidal cells from within the pyramidal layer of the CA3 sub-region in VPA group

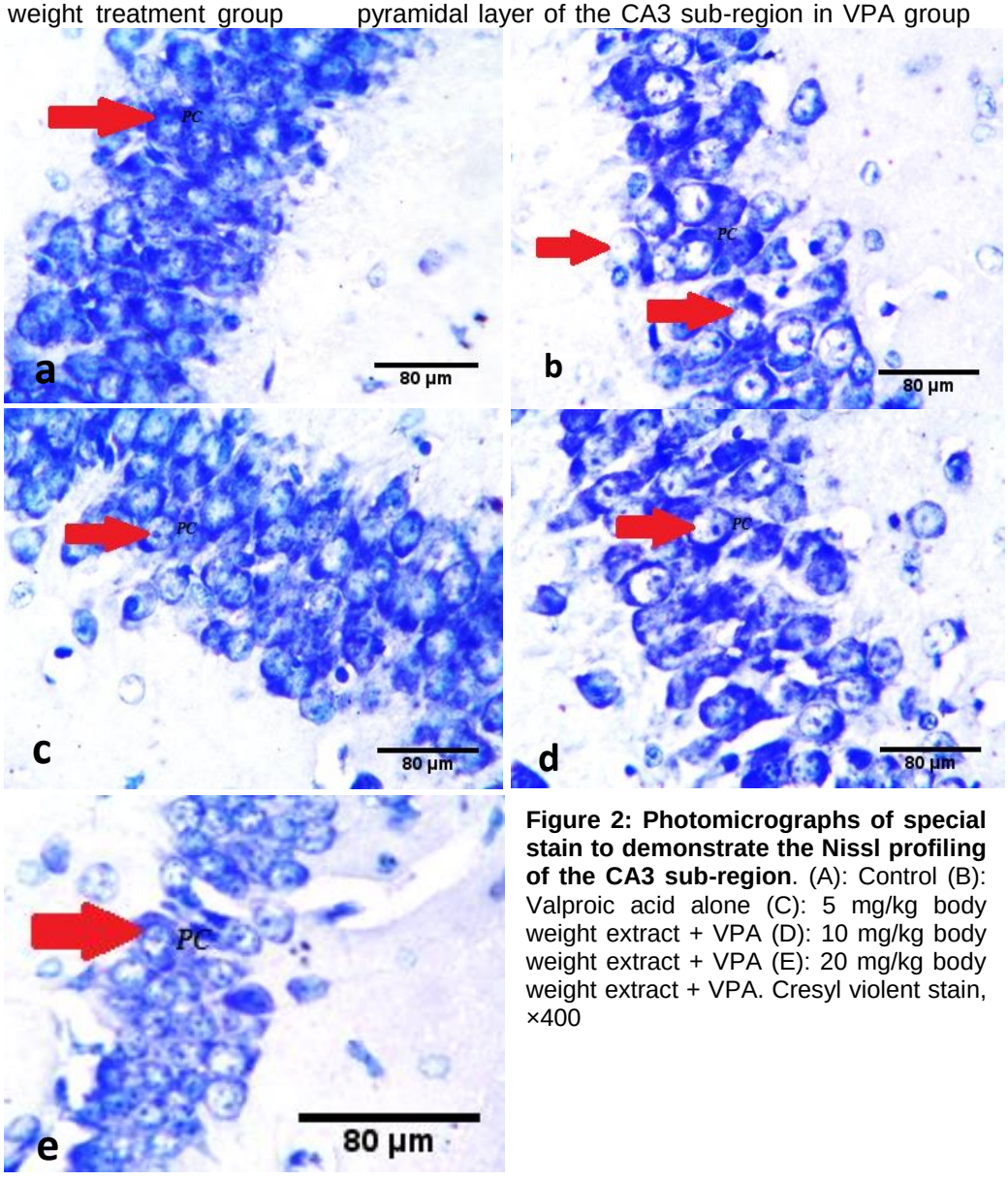

Figure 2: Photomicrographs of special stain to demonstrate the Nissl profiling of the CA3 sub-region. (A): Control (B): Valproic acid alone (C): 5 mg/kg body weight extract + VPA (D): 10 mg/kg body weight extract + VPA (E): 20 mg/kg body weight extract + VPA. Cresyl violent stain, ×400

when compared to the control group. However, there was an improvement in treatment groups 5, 10 and 20 mg/kg body weight; the pyramidal cell structure appeared better delineated from the surrounding layers and also presented a better stained cytoplasm than that of the VPA group. These positive features were more prominent in 5 mg/kg body weight treatment group when compared to the other groups. Nissl stained sections showed the appearance of chromatolysis in VPA group when compared to control group. This chromatolytic appearance seemed to be less prominent across the *C. longa* treated groups. A number of researchers have reported that hippocampal cell alterations are strong indications of the autistic disorder (Schumann et al. 2004; Takano 2015). These authors adduced that the presence of any alterations whatsoever may have debilitating effects on the hippocampal spatial recognition centre (CA3). Existing data emanating from a study on the effect of *C. longa* on the hippocampus in diabetic-induced rats has shown that curcumin demonstrates neuroprotective properties through its ability to preserve most of the pyramidal cells of the CA1, CA3 and dentate gyrus of the hippocampus (Faheem and El Askary 2017). In another study, curcumin exerted its neuroprotective role by attenuation of neuronal cell death in the hippocampal region thus, aiding better memory by promoting hippocampal long term potentiation ability (Sarona et al. 2018). The result of our study is in agreement with the report of these studies in which ethanolic extract of *C. longa* brought about an improvement in the morphologic appearance and delineation of the cells of the hippocampus as demonstrated in the photomicrographs.

Neurobehavioral studies with the elevated plus maze was used to analyse the level of anxiety in the test subjects. The anxiety level was determined based on the number of entries and time spent in the open arm of the maze. The present study showed no significant difference in the total number of entries into both the open and closed arms of the maze across all groups except the 20 mg/kg body weight treatment group. Worthy of note in this study, is the fact that, open arm time is considered to include both the time spent in the centre area and the time spent in the entrance into the exposed sides of the maze. The 5 mg/kg body weight treatment group had fewer entries into both arms when compared to the 10 mg/kg body weight treatment group which had the greatest number of entries into the closed arm in all the groups. A significant decrease was recorded in the time spent in open arm when compared to the time spent in the closed arm in the VPA, 10, and 20 mg/kg body weight treatment groups. The result of this study is somewhat similar to the study on postnatal administration, with similarity in entries into the open arms across other groups apart from the VPA-induced autism group which spent lesser time and walked less in the open arms (Lucchina and Depino 2014). The only contradiction in our study was observed in the less exploration time in the open arm demonstrated in the VPA group, as well as the 10 and 20 mg/kg body weight treatment groups. However, there was better exploration of the open arm by the 5 mg treatment group as deduced from the time spent in the open arm, though the number of entries was limited when compared to other groups. This illustrates some level of anti-anxiety in the 5 mg group as stated in a previous study (Walf and Frye 2007). They soon concluded that an increased open arm activity (duration or entries) exhibited anti-anxiety activity.

Another behavioural study in this research was the Crawley's sociability and test for social novelty. It aimed at exploring the interaction level of the test subject(s) by means of a three- chambered maze. All groups exhibited a preference for social interaction during the first stage of the test. Besides control group rats, all other groups demonstrated some level of preference for social novelty, by having a quantifiable number of entries into the chamber with stranger 2 during the second stage of the test. This illustrates a higher increase in interaction amongst the other groups, which seems to be more in VPA group. This data indicates that VPA group had more exploration of either chamber during the first stage of the test as depicted from its least number of entries and least duration spent in either chamber during the first stage of the test. However, more time was spent during the second stage with stranger 2 and there was equal number of active cusps contact when compared to other groups. This implies some level of hyperactivity, one of the comorbidities associated with ASD and inability to distinguish spatial environment among the VPA group. This contradicts another study (Moy et al 2004) in which control mice in-breeds showed a preference for the chamber with stranger 1 during the first session but switched to having a stranger 2 preference in the second session. However, similarity in the report of this study exists on the social interaction test (Lucchina and Depino 2014). They reported same levels of locomotive exploration by all test subjects in both the habitation and social interaction stages.

For the oxidative stress markers; reduced GSH, SOD, MDA and CAT were analysed. MDA levels recorded significant increase in the VPA and 20 mg/kg body weight treatment groups, while the 5 and 10 mg/kg body weight treatment groups recorded values comparable to control. Increase in MDA, an end product of lipid peroxidation is indicative of oxidative stress. This is consistent with a previous result (Chauhan et al. 2004), who associated oxidative stress to increased plasma levels of MDA. Oxidative stress occurs as a result of imbalance between the productions of reactive oxygen species (ROS) and antioxidant enzymes. Oxidative stress damages cells, by modifying proteins, lipids and DNA. The morphological alterations in the

hippocampus may be as a consequence of oxidative stress. The treated groups had better GSH values (comparable to control group) as they may have been able to mop up the excessive production of ROS which was confirmed by significant increase in MDA values in the VPA and 20 mg/kg body weight treatment groups. Similar reduction in levels of lipid peroxidase was observed in all curcumin treated groups (González-Fraguela et al. 2013; Al-Askar et al. 2017). This again affirms the antioxidant activity of curcumin; which is the main antioxidant component of the extract used in the present study. There was no significant change in the SOD level across all groups. Frustaci et al. (2012) reported a similar result when no association was found between SOD and ASD. The GSH biomarker indicated a significantly higher increase in VPA group when compared to the other groups. Worthy of note here is that there is a complementary action of either GSH or catalase to the function of SOD; as SOD aids the dismutation of superoxide radicals to H_2O_2, which is further detoxified to water and oxygen by CAT or GSH (Fitri et al. 2016). The increased level of GSH observed in this study is in response to the overproduction of ROS. Previous researchers have indicated that such rise in ROS production relates to a corresponding decrease in GSH (Frustaci et al. 2012; González-Fraguela et al. 2013; Al-Askar et al. 2017). However, a high GSH level has also been shown to play a dual role in mediating both physiological and pathological roles that cause drug resistance (Bansal and Simon 2018). Such treatment resistance has been seen in autistic individuals (Hellings et al. 2017). This increased GSH level in VPA group may account for the reduced compensatory antioxidant response to inflammation by CAT enzyme in this group (González-Fraguela et al. 2013). Although, the 10 mg/kg body weight treatment group indicated a significant increase in CAT when compared to VPA group, this is indicative of a possible impairment in ROS scavenging activity in the 10 mg/kg body weight group (Xie et al. 2018). A reduction in CAT level is another marked symptom seen in autistic individuals (Abdel-Salam et al 2015); however, VPA induced rodents showed no significant change in CAT levels in a previous study (Morakotsriwan et al. 2016). Contrary data illustrating an increased CAT level has been reported by another researcher (Altun et al. 2018). Data from the present study showed a different form of modification in the homeostasis of GSH which was ignited in VPA group as opposed to the other treatment and control groups. This increased build-up and accumulation of GSH following increase in ROS/free radicals depicts a significant continual detoxification activity in the VPA group. Though not significant, this increase in GSH was also slightly exhibited by the 20 mg/kg body weight group. Hence, these results show a dose-dependent decrease in response of oxidative markers in the treatment groups to the extract; as the group which received the low dose had results comparable to the control group.

Inflammatory cytokines have been shown to be good biomarkers in ASD. The pro-inflammatory cytokine; Interleukin-6 (IL-6) and anti-inflammatory cytokine; transforming growth factor (TGF β1) were analysed. Immune activation is manifested as increase in pro-inflammatory cytokines, which has been described in mild depression (Anderson and Maes 2014). A decrease in TGFβ1 level could reflect a role for cytokine imbalance affecting early neurodevelopment and the generation of clinical symptoms in ASD, as a result of immune dysfunction (Al-Askar et al. 2017). Such dysfunction is the case in the present study which indicated a significant decrease in TGF β1 in the 20 mg/kg body weight treatment group alone when compared to other groups. Although a rise in IL-6 may have been implied as a good indication for dysregulated immune response (Suzuki et al. 2011), there was no significant change across all groups, the VPA group inclusive. However, when compared with the control group, there were indications that the administration of the extract may have stabilized the cytokine levels in the other treatment groups to levels similar to the control. A previous research (Wei et al. 2011) related autism to an increase in IL-6 levels. However, the antioxidant capacity of the curcumin constituent of the administered extract in this study exhibits a significant lowering effect on IL-6 levels in cases of greater inflammation (Derosa et al. 2016). This suggests that there was either slight or no detectable localized molecular inflammation in the hippocampus, which would have ignited a significant activation of IL-6 in the subject pups. Furthermore, as described in the oxidative stress markers above, the 5 mg/kg body weight treatment group exhibited much closer values to the control than the other treatment groups in both the anti and pro-inflammatory cytokines.

Dopaminergic signalling is established as playing an important role in novelty related modulation of hippocampal memory (McNamara and Dupret 2017). There is evidence to demonstrate the role of dopamine in neuronal plasticity, synaptic transmission and the network activity in the hippocampal circuitry underlying spatial memory (McNamara et al. 2014; Rosen et al. 2015). Disruptions and subsequent mutations in dopamine transporters or receptors have been associated with manifestation of the core symptoms of autism-like behaviours (Hamilton et al. 2013). The dopamine levels in this study showed no significant difference across all groups. However, results from this present study may therefore imply that the hippocampus may just be passively involved in the mesocorticolimbic diffuse dopaminergic circuit. This circuit is a diffuse modulatory system of neurons (ventral tegmental area and substantia nigra) from the midbrain, which form broad connections scattered throughout the brain (Pavăl 2017). Notwithstanding, relating the

essential role of dopamine in regulating behaviour, show an insignificant elevation in dopamine levels in both the 10 and 20 mg/kg body weight treatment groups. This accounts for the increased anxiety state, similar to VPA group and overly active social exploration in these groups when compared to the control and 5 mg/kg body weight treatment groups.

Conclusively, the improved morphological appearance of sections of the hippocampus and improved social behaviour observed in this study, are indications that the prenatal administration of ethanolic extract of *C. longa* as a supplement throughout pregnancy could be a useful ameliorative agent in ASD. We recommend future studies to explain the *in vivo* mechanism of action of this extract.

Conflict of Interest

None declared.

Acknowledgement

We acknowledge Dr. A. Nnaemeka, of the Central Research Laboratory, Tanke Ilorin, Nigeria, for the biochemical analysis. Also, our appreciation goes to Dr O.A. Salako and Dr G.O. Afolayan of the Department of Pharmacology, and Mr. O. Yusuf, a technologist of the Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria. They all assisted with the plant extraction and quantification.

REFERENCES

Abdel-Salam, O.M., Youness, E.R., Mohammed, N.A. and Elhamed, W.A.A. (2015) Nuclear factor-kappa B and other oxidative stress biomarkers in serum of autistic children. Open Journal of Molecular and Integrative Physiology. 5(1):18.

Al-Askar, M., Bhat, R.S., Selim, M., Al-Ayadhi, L. and El-Ansary, A. (2017) Postnatal treatment using curcumin supplements to amend the damage in VPA-induced rodent models of autism. BioMed Central Complementary and Alternative Medicine. 17(1):259.

Altun, H., Şahin, N., Kurutaş, E.B., Karaaslan, U., Sevgen, F.H. and Fındıklı, E. (2018) Assessment of malondialdehyde levels, superoxide dismutase, and catalase activity in children with autism spectrum disorders. Psychiatry and Clinical Psychopharmacology. 28(4):408-415.

Anderson, G. and Maes, M. (2014) Redox regulation and the autistic spectrum: role of tryptophan catabolites, immuno-inflammation, autoimmunity and the amygdala. Current Neuropharmacology. 12(2):148-167.

Bagchi, A. (2012) Extraction of curcumin. IOSR Journal of Environmental Science, Toxicology and Food Technology. 1(3):1-16.

Bakare, M.O., Ebigbo, P.O., Agomoh, A.O., Eaton, J., Onyeama, G.M., Okonkwo, K.O. et al. (2009) Knowledge about childhood autism and opinion among healthcare workers on availability of facilities and law caring for the needs and rights of children with childhood autism and other developmental disorders in Nigeria. BioMed Central Pediatrics. 9(1):12.

Bakare, M.O., Ebigbo, P.O. and Ubochi, V N. (2012) Prevalence of autism spectrum disorder among Nigerian children with intellectual disability: a stopgap assessment. Journal of Health Care for the Poor and Underserved. 23(2):513-518.

Bang, M., Lee, S.H., Cho, S.-H., Yu, S., Kim, K., Lu, H.Y. et al. (2017). Herbal medicine treatment for children with autism spectrum disorder: a systematic review. Evidence-Based Complementary and Alternative Medicine. 2017.

Bansal, A. and Simon, M.C. (2018) Glutathione metabolism in cancer progression and treatment resistance. Journal of Cell Biology. 217(7):2291-2298.

Chauhan, A., Chauhan, V., Brown, W.T. and Cohen, I. (2004) Oxidative stress in autism: Increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin-the antioxidant proteins. Life Sciences. 75(21):2539-2549.

Choi, C.S., Gonzales, E.L., Kim, K.C., Yang, S.M., Kim, J.-W., Mabunga, D.F. et al. (2016). The transgenerational inheritance of autism-like phenotypes in mice exposed to valproic acid during pregnancy. Scientific Reports. 6:36250.

Courchesne, E., Pierce, K., Schumann, C.M., Redcay, E., Buckwalter, J.A., Kennedy, D.P. et al. (2007) Mapping early brain development in autism. Neuron. 56(2):399-413.

Cox, K.H., Pipingas, A. and Scholey, A.B. (2015). Investigation of the effects of solid lipid curcumin on cognition and mood in a healthy older population. Journal of Psychopharmacology. 29(5):642-651.

Derosa, G., Maffioli, P., Simental-Mendia, L.E., Bo, S. and Sahebkar, A. (2016) Effect of curcumin on circulating interleukin-6 concentrations: a systematic review and meta-analysis of randomized controlled trials. Pharmacological Research. 111:394-404.

Faheem, N.M. and El Askary, A. (2017). Neuroprotective role of curcumin on the hippocampus against the structural and serological alterations of streptozotocin-induced diabetes in Sprague Dawley rats. Iranian Journal of Basic Medical Sciences. 20(6):690.

Fitri, L.E., Iskandar, A., Sardjono, T.W., Erliana, U.D., Rahmawati, W., Candradikusuma, D. et al. (2016) Plasma glutathione and oxidized glutathione level, glutathione/oxidized glutathione ratio, and albumin concentration in complicated and uncomplicated falciparum malaria. Asian Pacific Journal of Tropical Biomedicine. 6(8):646-650.

Franz, L., Chambers, N., von Isenburg, M. and de Vries, P.J. (2017) Autism spectrum disorder in

sub-saharan africa: A comprehensive scoping review. Autism Research. 10(5):723-749.

Frustaci, A., Neri, M., Cesario, A., Adams, J.B., Domenici, E., Dalla Bernardina, B. et al. (2012) Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. Free Radical Biology and Medicine. 52(10):2128-2141.

Gbotolorun, S., Oremosu, A., Osinubi, A., Noronha, C., Coker, H. and Silva, B. (2012) Ameliorative effect of Vitamin E on the deleterious effect of amodiaquine hydrochloride (AQ. HCl) on the reproductive function of the adult cyclic Sprague-Dawley (SD) rats. Biology and Medicine. 4(3):141.

González-Fraguela, M.E., Hung, M.-L.D., Vera, H., Maragoto, C., Noris, E., Blanco, L. et al. (2013) Oxidative stress markers in children with autism spectrum disorders. Journal of Advances in Medicine and Medical Research. 307-317.

Hamilton, P., Campbell, N., Sharma, S., Erreger, K., Herborg Hansen, F., Saunders, C. et al. (2013) Consortium NAAS, Sahai MA, Cook EH, Gether U, McHaourab HS, Matthies HJ, Sutcliffe JS, Galli A (2013) De novo mutation in the dopamine transporter gene associates dopamine dysfunction with autism spectrum disorder. Molecular Psychiatry. 18: 1315-1323.

Hellings, J.A., Arnold, L.E. and Han, J.C. (2017) Dopamine antagonists for treatment resistance in autism spectrum disorders: review and focus on BDNF stimulators loxapine and amitriptyline. Expert Opinion on Pharmacotherapy. 18(6):581-588.

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J. and Woodgett, J. R. (2011) Assessment of social interaction behaviors. JoVE (Journal of Visualized Experiments). (48):e2473.

Karim, M.R., Haque, A., Islam, K., Ali, N., Salam, K.A., Saud, Z.A. et al. (2010) Protective effects of the dietary supplementation of turmeric (Curcuma longa L.) on sodium arsenite-induced biochemical perturbation in mice. Bangladesh Medical Research Council Bulletin. 36(3):82-88.

Kataoka, S., Takuma, K., Hara, Y., Maeda, Y., Ago, Y. and Matsuda, T. (2013) Autism-like behaviours with transient histone hyperacetylation in mice treated prenatally with valproic acid. International Journal of Neuropsychopharmacology. 16(1):91-103.

Landau, B. (2017) Update on "what and where" in spatial language: A new division of labor for spatial terms. Cognitive Science. 41(S.2):321-350

LeClerc, S. and Easley, D. (2015) Pharmacological therapies for autism spectrum disorder: a review. Pharmacy and Therapeutics. 40(6):389.

Li, G., Cheng, M-H., Li, G., Wu, D., Sun, Q., Shen, D. et al. (2019) A preliminary volumetric MRI study of amygdala and hippocampal subfields in autism during infancy. IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019), Venice. pp.1052-1056. Doi: 10.1109/ISBL.2019.8759439.

Liu, D., Schwimer, J., Liu, Z., Woltering, E.A. and Greenway, F.L. (2008) Antiangiogenic effect of curcumin in pure versus in extract forms. Pharmaceutical Biology. 46(10-11): 677-682.

loomes, R., Hull, Laura., Polmear, W. and Mandy, L. (2017) What is the male-to-female ratio in austism spectrum disorder? a systematic review and meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 56(6):466-474.

Lucchina, L. and Depino, A.M. (2014) Altered peripheral and central inflammatory responses in a mouse model of autism. Autism Research. 7(2):273-289.

Marklund, S. and Marklund, G. (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 47(3):469-474.

McNamara, C.G. and Dupret, D. (2017) Two sources of dopamine for the hippocampus. Trends in Neurosciences. 40(7):383-384.

McNamara, C. G., Tejero-Cantero, Á., Trouche, S., Campo-Urriza, N. and Dupret, D. (2014) Dopaminergic neurons promote hippocampal reactivation and spatial memory persistence. Nature Neuroscience. 17(12):1658.

Morakotsriwan, N., Wattanathorn, J., Kirisattayakul, W. and Chaisiwamongkol, K. (2016) Autistic-like behaviors, oxidative stress status, and histopathological changes in cerebellum of valproic acid rat model of autism are improved by the combined extract of purple rice and silkworm pupae. Oxidative Medicine and Cellular Longevity, 2016.

Moy, S., Nadler, J., Perez, A., Barbaro, R., Johns, J., Magnuson, T. et al. (2004) Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes, Brain and Behavior. 3(5):287-302.

Nicolini, C. and Fahnestock, M. (2018) The valproic acid-induced rodent model of autism. Experimental Neurology. 299: 217-227.

Oshodi, Y.O., Olagunju, A.T., Oyelohunnu, M.A., Campbell, E.A., Umeh, C.S., Aina, O.F. et al. (2016) Autism spectrum disorder in a community-based sample with neurodevelopmental problems in Lagos, Nigeria. Journal of Public Health in Africa. 7(2).

Patterson, P.H. (2011) Modeling autistic features in animals. Pediatric Research. 69(5):34R-40R.

Paul, C.A., Beltz, B. and Berger-Sweeney, J. (2008) The Nissl stain: a stain for cell bodies in brain sections. CSH protocols, 2008,pdb.prot4805. doi:10.1101/pdb.prot4805

Pavăl, D. (2017) A dopamine hypothesis of autism spectrum disorder. Developmental Neuroscience. 39(5): 355-360.

Quinlan, T., Spivack, S. and Mossman, B. (1994) Regulation of antioxidant enzymes in lung after oxidant injury. Environmental Health Perspectives. 102(suppl 2): 79-87.

Rosen, Z.B., Cheung, S. and Siegelbaum, S.A. (2015) Midbrain dopamine neurons bidirectionally

regulate CA3-CA1 synaptic drive. Nature Neuroscience. 18(12): 1763.

Rukkumani, R., Aruna, K., Varma, P.S., Rajasekaran, K.N. and Menon, V.P. (2004) Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. Journal of Pharmacy and Pharmaceutical Sciences. 7(2):274-283.

Sarona, J.E.E.P., Ples, M.B. and Vitor II, R.J.S. (2018) Neurotrophic effects of turmeric on the memory of the mouse using the Morris water maze test. National Journal of Physiology, Pharmacy and Pharmacology. 8(5):726-731.

Schumann, C.M., Hamstra, J., Goodlin-Jones, BL., Lotspeich, L.J., Kwon, H., Buonocore, M.H. et al. (2004) The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. Journal of Neuroscience. 24(28):6392-6401.

Suzuki, K., Matsuzaki, H., Iwata, K., Kameno, Y., Shimmura, C., Kawai, S. et al. (2011) Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. PloS one. 6(5):e20470.

Takano, T. (2015) Interneuron dysfunction in syndromic autism. Recent Advances. Developmental Neuroscience. 37(6):467-475.

Tejada, J., Bosco, G.G., Morato, S. and Roque, A.C. (2009). Characterization of rat behavior in the elevated plus-maze using a directed graph. Journal of Neuroscience Methods. 184(2):251-255.

van Gelder, M.M., Roeleveld, N. and Nordeng, H. (2011) Exposure to non-steroidal anti-inflammatory drugs during pregnancy and the risk of selected birth defects: a prospective cohort study. PloS ONE. 6(7):e22174.

Walf, A.A. and Frye, C.A. (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols. 2(2):322.

Wei, H., Zou, H., Sheikh, A.M., Malik, M., Dobkin, C., Brown, W.T. et al. (2011) IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. Journal of Neuroinflammation. 8(1):52.

WHO (2012) Guideline: daily iron and folic acid supplementation in pregnant women: Geneva, World Health Organization. http://www.who.int/nutrition/publications/micronutrients/supp_pregnant_women/en/).

Wiens, D. and DeSoto, M.C. (2017) Is high folic acid intake a risk factor for autism?—a review. Brain Sciences. 7(11):149.

Won, H., Mah, W. and Kim, E. (2013) Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. Frontiers in Molecular Neuroscience. 6:19.

Wright, A., Hawkins, C.L. and Davies, M.J. (2003) Photo-oxidation of cells generates long-lived intracellular protein peroxides. Free Radical Biology and Medicine. 34(6):637-647.

Xie, Z., Wu, B., Shen, G., Li, X. and Wu, Q. (2018) Curcumin alleviates liver oxidative stress in type 1 diabetic rats. Molecular Medicine Reports. 17(1):103-108.

Xu, Y., Ku, B., Cui, L., Li, X., Barish, P.A., Foster, T.C. et al. (2007) Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Research. 1162:9-18.

Cite as: Gbotolorun, S.C., Okoh, C.O.A. and Oshodi, Y.O. (2020) Prenatal supplementation with Curcuma longa ameliorates oxidative stress, improves behaviour and hippocampal alterations in valproic acid-induced autism in Sprague-Dawley rat. Nig. J. Neurosci. 11(1):8-20. http://doi.org/10.47081/njn2020.11.1/002