

Original Research Article

Crassocephalum crepidioides extracts reverses the effects of valproic acid in an *in-utero* mouse model of autistic disorders

Irene Alice Folefack¹, Sefirin Djiogue^{2*}, Jean G. Beppe¹, Nanou Gael Allah-Doum¹, Chiara N. Adjoffoin², Rudig Nikanor T. Djikem², Bertrand M. Barga¹, Perpetue M. Atsama², Woudamkine Sadou¹, Elisabeth Ngo Bum¹

¹Faculty of Science, University of Maroua, Maroua, Cameroon

²Faculty of Science, University of Yaounde I, Yaounde, Cameroon

Received: 26 December 2024

Revised: 29 January 2025

Accepted: 15 April 2025

*Correspondence:

Dr. Sefirin Djiogue,

E-mail: sefirin.djiogue@facsciences-uy1.cm

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by deficits in social communication and interaction, alongside restricted or repetitive behaviours. This study investigates the potential of hydroethanolic extract of *Crassocephalum crepidioides* (HEE) to ameliorate ASD-related behaviours in a valproic acid (VPA)-induced mouse model of *in utero* ASD. VPA exposure disrupts neurodevelopmental processes, affecting synaptic function and neural connectivity which are also seen in Autism.

Methods: Thirty pregnant female mice were used among which, six served as normal control group. the twenty-four remaining mice received valproic acid 600 mg/kg on gestational day 10 to day 12. Following parturition, offspring were treated daily from postnatal day 21 to 50. Behavioural assessments were conducted during the treatment period. Upon completion of behavioural testing, brains were collected for biochemical analysis of monoamine levels.

Results: Compared to the VPA-exposed control group, HEE treatment at all tested doses significantly reduced ($p < 0.001$) the time spent investigating an empty chamber in a social preference test, and significantly increased ($p < 0.001$) the time spent interacting with a social stimulus (sentinel). Furthermore, HEE treatment significantly increased ($p < 0.001$) brain tissue concentrations of monoamines.

Conclusions: These findings suggest that *Crassocephalum crepidioides* extract may exert neuroprotective effects, improving both social behaviour and reducing repetitive behaviours in this VPA-induced mouse model of ASD. HEE has therapeutic potential by modulating neurotransmitter systems known to be disrupted in Autism.

Keywords: Autism spectrum disorder, Valproic acid, *Crassocephalum crepidioides*, Neuroprotection, Social behaviour

INTRODUCTION

Autism spectrum disorder (ASD) is a set of disorders characterized by deficiency of social behaviour, communication and language alterations, and a limited set of interests and activities that are both unreciprocated and directed repetitively.¹ It affects 1 to 2% of people worldwide.² According to the epidemiological

study,³ autism accounts for approximately one third of pervasive developmental disorders. Autism is a multifactorial disorder with a genetic predominance. The etiology of autism currently remains extremely complex and poorly understood. In 5 to 10% of cases, there is a comorbidity associated with another pathology, such as Down's syndrome or fragile X.⁴ Several risk factors contribute to the onset of ASDs. It involves both genetic

and epigenetic factors, i.e. the influence of the environment on gene expression, most often through exposure to environmental factors *in utero*.⁵ The use of certain substances by women during pregnancy exposes their offspring to autism spectrum disorders, such as valproic acid. Valproic acid is a neuroleptic widely used for its proven efficacy in epilepsy, but it remains the most toxic to prenatal development. Administration of valproic acid (VPA) to pregnant rodents at a critical time is a widely used animal model of ASD.⁶ Valproate indirectly potentiates GABA by increasing its synaptic concentration and thus its effects; this inhibitory mediator of the central nervous system in adult mammals is, however, excitatory and neurotrophic during embryonic life.⁶ The diversity of risk factors for autism further complicates its treatment, especially as there is as yet no cure. Caring for people with autism can improve both their quality of life and that of their families. Several studies have demonstrated the effectiveness of behavioural and psychosocial interventions as a first-line treatment for ASD symptoms.⁷ Current therapeutic options, such as psychosocial approaches and synthetic drugs, are limited in their ability to improve neural function or regeneration.⁷ Several classes of drugs are used in the treatment of autistic patients : antipsychotics to improve maladaptive behaviour ; serotonin reuptake inhibitors to relieve depressive, mood and obsessive-compulsive disorders (OCD) ; anxiolytics such as benzodiazepines ; and anticonvulsants which act by reinforcing the protective action of GABA.⁸ These treatments have undesirable effects such as vomiting, ulcerations, hallucinations and diarrhoea, as well as being incapable of permanently curing ASD. To cope with these limitations, people are increasingly turning to alternative treatments based on natural plants to relieve autism spectrum disorders.⁹ Plants containing phenolic compounds have potentially neuroprotective effects and can be used to treat psychological or neurological disorders due to their antioxidant properties.^{10 11} Showed that *Bacopa monniera* wehst improved behavioral disorders and oxidative stress in a valproic acid autism model in rats. The anxiolytic and antidepressant effects of *Silvia* spp. essential oil were demonstrated by ¹² in a rat model of autism spectrum disorder. To make our contribution to this development of phytotherapy for autism spectrum disorders, we focused on *Crassocephalum crepidioides*, a herbaceous plant in the Asteraceae family. *Crassocephalum crepidioides* is traditionally used to treat epilepsy, headaches and wounds in Nigeria and Cameroon.¹³ A study on the anti-inflammatory, immunomodulatory and anti-genotoxic potential of *Crassocephalum crepidioides* methanolic extract was done by.¹⁴ A phytochemical and toxicity study of *Crassocephalum crepidioides*¹³ and an in vitro study of its anti-cholinesterase activity¹⁵ have been carried out. However, to our knowledge, no scientific study of this plant in the improvement of autistic disorders has yet been conducted. Hence the interest of this work, the general aim of which was to develop the properties of the hydroethanolic extract of *Crassocephalum crepidioides*

leaves on some autistic disorders induced in utero by valproic acid in mice.

METHODS

Materials

Chemical substances

The valproic acid (Depakine) and Risperidone (Risperdal) used in this study were obtained from Sigma Aldrich (USA) and dissolved in saline (0.09%) and distilled water respectively. These solutions were freshly prepared and administered orally.

Plant material

Fresh plants of *Crassocephalum crepidioides* (Asteraceae) were harvested in January 2022, was identified and authenticated at the National Herbarium by comparison with a sample held there under reference number NHC 24250/SRF.

Animal material

Thirty female mice were used in this experiment. These animals were purchased in advance from the national veterinary laboratory in Garoua (LANAVET). They were housed in plastic cages and acclimatized for a fortnight before the start of the experiment in the Laboratory of Animal Physiology and Pharmacognosy of the University of Maroua.

Methods

Extraction protocol

Fresh *Crassocephalum crepidioides* (Cc) plants were shade-dried and ground. 500 g of the powder obtained was macerated in 3 liters of water/ethanol (v/v :20/80) for 72 hours at room temperature. The macerate obtained was then filtered through whatman paper. The filtrate obtained was concentrated in a BUCHI rotavapor (R-300) at 60°C and then dried in an oven at 50°C for 48 hours. A dry extract (47 g) was obtained, giving an extraction yield of 9.4%.

Evaluation of antioxidant activity in-vitro

DPPH and FRAP

Assessment of reducing power by FRAP

The FRAP test was performed using the method described.¹⁶ The method consisted on the capacity of an antioxidant to reduce the Fe(III)-2,4,6-Tri (2-pyridyl-s-triazine (TPTZ) complex to a blue Fe(II)-TPTZ complex that exhibits an absorption maximum at 593 nm. Results were determined using the optical density calibration

curve as a function of Trolox concentration (0-140 µg/ml) and (mg ET/100g).

$$\text{FRAP} \left(\frac{\text{mgET}}{100\text{g}} \text{MS} \right) = \frac{\text{DO} \times \text{fd} \times \text{V}}{\text{Pexa}}$$

With TFRAP = Reducing capacity of iron in FRAP; OD = Optical density; fd = Dilution factor; V=Total extraction volume; Pe =Test sample; a = Slope of calibration curve.

Evaluation of the anti-free radical activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH- radical scavenging activity was determined using the method described.¹⁷ 150 µl of sample extract or standard solution (Trolox 10 mg/ml) was added to 1500 µl of DPPH- (1mM) in a 10 ml screw tube. The mixture was shaken for 60 seconds and incubated for 30 minutes in the dark at 35°C. The absorbance at 517 nm of the mixture was read.

$$\text{DPPH} \left(\frac{\text{mgET}}{100\text{g}} \text{MS} \right) = \frac{\text{DO} \times \text{fd} \times \text{V}}{\text{Pexa}}$$

With DPPH = Deprotection capacity; DO = Optical density; fd = Dilution factor; V=Total extraction volume; Pe =Test sample; a = Slope of calibration curve.

Animals treatment

The present study is a transversal study. The experiments took place from June 2023 to August 2023. Thirty pregnant mice were randomly divided into 2 groups: the normal control group of 6 animals received saline (0.9%, p.m.); and the control group of 24 animals received sodium valproate (600 mg/kg, p.o.) from day 9 to 11 of gestation. After parturition, the mice in the control group were divided into 5 groups (Table 1) on day 21 of life, and only the male mice that had manifested the disease were retained for further treatment. The normal control male mice remained in the normal group. Behavioural testing began after 30 days of treatment.

Table 1: Treatment distribution of animals on day 21 of life.

Groups	Treatments	Doses and routes of administration
Normal control	Distilled water	Normal 10 ml/kg, p.o
Negative control	Distilled water	10 ml/kg, p.o
Positive control	Risperidone	Risp 2 mg/kg, p.o
Test 1	Extract	Cc 100 mg /kg, p.o
Test 2	Extract	Cc 200 mg /kg, p.o
Test 3	Extract	Cc 4 00 mg /kg, p.o

After all behavioural tests, the animals were sacrificed by diazepam/ketamine euthanasia (10 mg/Kg and 50 mg/Kg, i.p., respectively). Their brains were isolated, a hemisphere preserved in 10% formalin for histology. Another was homogenized in phosphate buffer for the evaluation of oxidative stress parameters: malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) as well as the determination of the concentration of serotonin, dopamine and GABA.

Behavioural tests

Sociability test

Sociability is the most studied behaviour in VPA models of autism. For this test, a rectangular cage is separated into 3 zones or chambers, leaving a passageway so that the test animal can easily move from one chamber to another.¹⁸ The mouse is initially placed in the empty middle chamber, to its left a conspecific mouse is placed in a restraint box that must leave space for interaction, and to the right, the same restraint box but empty. The sociability index will therefore be calculated by comparing the time spent interacting with the conspecific mouse or staying in the same room with the time spent exploring the empty box or the empty room.

Stereotyped movement test

Principle: The open arena is a test used to assess the exploratory abilities of mice in an aversive context and their emotional state when placed in a novel environment.¹⁹ This test is based on repetitive movements, on the animal's spontaneous exploratory behaviour and on the mouse's natural aversion to uncovered environments. The apparatus used in this work is an arena containing sawdust (litter).

Experimental procedure: all animal was initially placed in the middle of the maze and its behaviour observed for 5 minutes. The variables measured during this test were: 1) the latency time (s), which corresponds to the time taken to leave the centre of the arena; 2) total burrowing time (the animal digs sawdust with its snout or forepaws); 3) the total number of groomings;

Biochemical assays

Oxydative stress assays

Malondialdehyde (MDA) assay

MDA levels in the hippocampus were determined using the method described by the absorbance was read at 532 nm against the blank.²⁰ The quantities of MDA were evaluated and expressed as Mm/mg of organ.

Superoxide dismutase (SOD) assay

The SOD assay was performed according to the principle described, which is therefore based on the ability of SOD to inhibit or delay the auto-oxidation of adrenaline to adrenochrome in a basic medium.²¹ Autoxidation was measured by OD reading at 480 nm at t=30 seconds and at t=90 seconds. SOD activity was expressed in units/mg of organ.

Catalase assay

Catalase was assayed according to the method described.²² Optical densities were read at 570 nm.

Neurotransmitters assay

Determination of serotonin

The serotonin was assayed according to the method used by the absorbances were read at 470 nm using a spectrofluorometer against the blank.²³

Determination of dopamine

In the presence of hydrochloric acid, dopamine oxidises to give an indole derivative which combines with trihydroxyindoles to give a fluorescent complex whose absorbance at 485 nm is proportional to the concentration of dopamine.²⁴

Gamma aminobutyric acid assay

This assay is based on the fact that the reaction between ninhydrin and gamma aminobutyric acid (GABA) in a basic medium forms a complex whose absorbance is proportional to the amount of GABA in the sample.²⁵ The fluorescence resulting from the reaction between ninhydrin and GABA in the basic medium was measured using a spectrofluorimeter and was gradual to the concentration of GABA in the homogenates.

Histological study

The histological study technique used in this work is the basic technique described.²⁶ It consists successively of: fixation, macroscopy, dehydration, inclusion, sectioning, staining and mounting. The fixed tissues were first cleaned of all traces of water before they could be embedded in this medium due to the immiscibility of paraffin with water; Inclusion allowed microtome sections to be made by providing an external support for the tissues; a LEICA RM 2125 RT handwheel microtome was used to cut the blocks obtained, and the 5 µm-thick strips of sections obtained were spread out in a water bath containing gelatinised water. Using clean, labelled slides, the sections were collected and oven-dried at 45°C for 24 hours. The staining technique used was the standard haematoxylin-eosin staining technique for histological preparations; the slides were mounted to preserve staining; the coverslip was

adherent and the preparation was ready for microscopic observation. The microarchitecture of the hippocampus was observed under a microscope with a magnification of ×100 for the dentate gyrus and ×200 for Ammon's horns.

Statistical analysis

All analyses were performed using Graph Pad Prism version 8.0.1 for Windows. The results were expressed as mean ± MSE. Data were analysed by one-factor ANOVA and two-factor ANOVA followed by Dunnett's and Bonferroni's post-tests respectively. Results were considered significant for $p < 0.05$.

RESULTS

In-vitro essay of *Crassocephalum crepidioides* hydroethanol extract

The antioxidant potential of the hydroethanol extract (HEE) of Cc was measured using the DPPH and FRAP tests (Figure 1). The results of these two tests showed that the extract had a DPPH inhibition percentage of 74.13% and a FRAP inhibition percentage of 65.27% compared with the positive control (BHT), which had an inhibition percentage of 75.45%. Cc EHE had an inhibitory concentration 50 (IC₅₀) of 170µg/ml compared with the positive control, which had an inhibitory concentration (IC₅₀) of 150µg/ml (Figure 1).

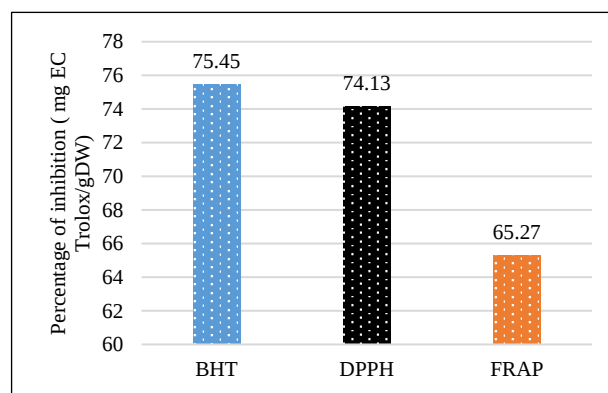


Figure 1: In-vitro antioxidant activity of hydroethanolic extract of *Crassocephalum crepidioides*.

Effect of *Crassocephalum crepidioides* hydroethanol extract on social behaviour in the single animal social interaction test

Figure 2 below presents the results on the time spent with the empty pen and the time spent with conspecifics (Figure 2A) of the animals; and also, on the number of contacts (Figure 2B) of these animals with the empty pen and conspecifics after 30 days of treatment. These results show that valproic acid subsequently ($p < 0.001$) reduced the time spent and the number of contacts with the conspecific in animals in the valproate fraction compared with animals in

the normal control class. Conversely, plant extract at all doses tested increased significantly ($p < 0.001$) in these parameters in mice in the valproate group contrasted with animals in the negative control group. Similarly, Risperidone induced a seriously increase ($p < 0.001$) in these parameters in animals contrasted with animals in the valproate faction (Figure 2).

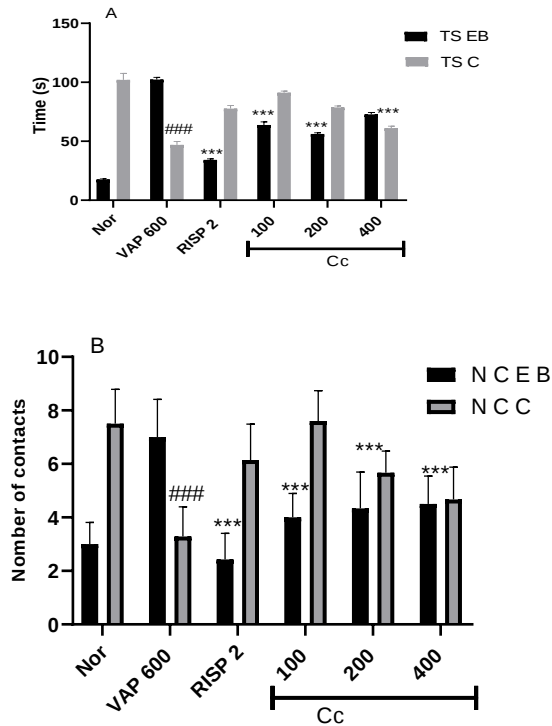


Figure 2: Effect of *Crassocephalum crepidioides* hydroethanol extract on social interaction in the one-animal sociability test.

Each band represents the mean $\pm$ MSE, ($n = 7$), *** $P < 0.001$ significant difference from negative control. #### $P < 0.001$ significant difference from normal control. VPA = valproic acid for negative control; RISP = risperidone for positive control; Cc = hydroethanol extract of *Crassocephalum crepidioides*.

Effect of *Crassocephalum crepidioides* extract on animal behaviour in the stereotyped movement test.

Evaluation of the burial time of the animals gave the results shown in Figure 3 below. Valproic acid induced a notable diminish ($p < 0.001$) in burying time in animals in the valproate class contrasted with animals in the normal control class. Conversely, administration of plant extract at all doses tested induced a notable diminish ($p < 0.001$) in burial time in animals compared with animals in the valproate faction. Similarly, Risperidone induced a important diminish ($p < 0.001$) in this parameter in animals contrasted with animals in the vaprote class (Figure 3).

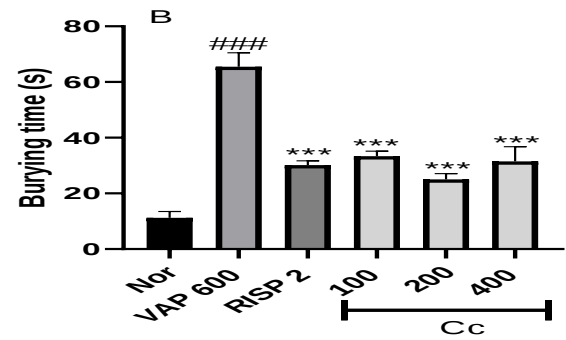


Figure 3: Effect of *Crassocephalum crepidioides* hydroethanol extract on stereotyped movements in the open arena test.

Each band represents the mean $\pm$ MSE, ($n = 7$), *** $P < 0.001$ significant difference from negative control. #### $P < 0.001$ significant difference from normal control. VPA = valproic acid for negative control; RISP = risperidone for positive control; Cc = hydroethanol extract of *Crassocephalum crepidioides*.

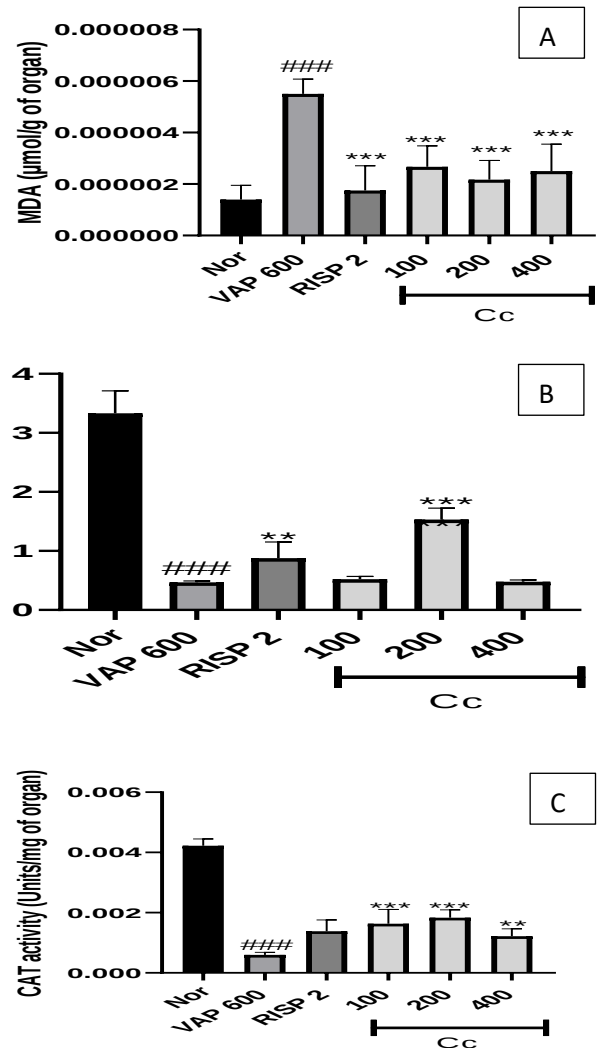


Figure 4 (A-C): Effect of hydroethanol extract of *Crassocephalum crepidioides* on some markers of oxidative stress.

Each band represents the mean $\pm$ MSE, (n = 7), **P < 0.001 significant difference from negative control, ***P < 0.001 significant difference from negative control, ###P < 0.001 significant difference from normal control. VPA = valproic acid for negative control; RISP = risperidone for positive control; Cc = hydroethanolic extract of *Crassocephalum crepidioides*.

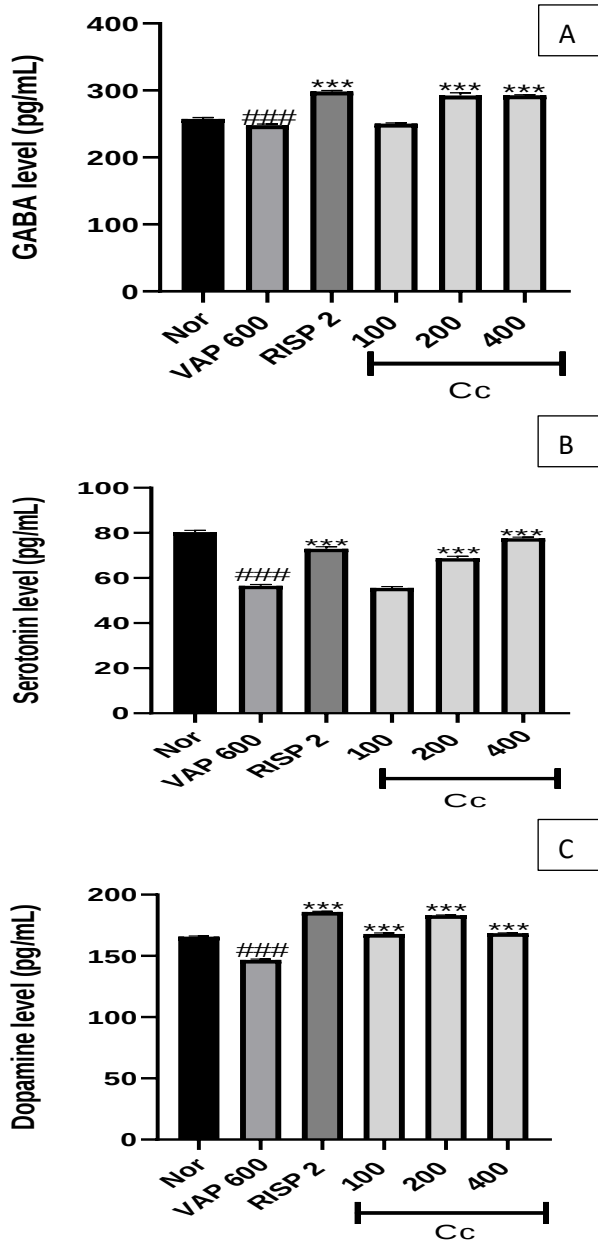


Figure 5 (A-C): Effect of hydroethanol extract of *Crassocephalum crepidioides* on the concentration of some neuromediators.

Each band represents the mean $\pm$ MSE, (n = 7), ***P < 0.001 significant difference compared with the negative control. ###P < 0.001 significant difference from normal control. VPA = Valproic acid for negative control; RISP = Risperidone for positive control; Cc = Hydroethanol extract of *Crassocephalum crepidioides*.

Effect of *Crassocephalum crepidioides* extract on some markers of oxidative stress.

Figure 5 below presents the results on the concentration of MDA (Figure 4A), the activity of SOD (Figure 4B) and the activity of CAT (Figure 4C) in the homogenate of the brains of mice after 30 days of treatment. Valproic acid induced a significant increase ($p < 0.001$) in MDA concentration and a significant decrease ($p < 0.001$) in SOD and CAT activity in animals in the valproate group compared with animals in the normal control group. Conversely, plant extract significantly ($p < 0.001$) decreased MDA concentration at all doses tested, significantly ($p < 0.001$) increased SOD activity at 200 mg/kg and significantly (100 and 200 mg/kg, $p < 0.001$; 400 mg/kg, $p < 0.01$) increased CAT activity in all animals compared with animals in the valproate group. Risperidone induced a significant decrease ($p < 0.001$) in MDA levels and a significant increase ($p < 0.01$) in SOD activity (Figure 4).

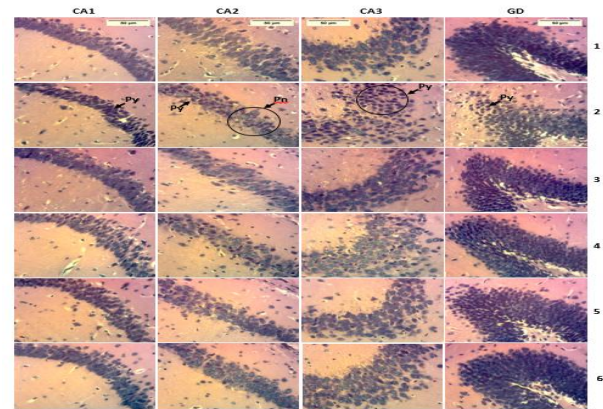


Figure 6: Microphotographs of CA1, CA2, CA3 areas and the dentate gyrus of the mouse hippocampus ; stained with haematoxylin-eosin.

1 = Normal control; 2 = Negative control; 3 = Positive control; 4, 5 and 6 = Batches receiving Cc extract at the doses of 100, 200 and 400mg/kg; Ne = Neuron; Py = Pycnosis; Cv = Vacuolised cells.

Effect of *Crassocephalum crepidioides* extract on the concentration of some neurotransmitters.

Figure 5 below shows the results on the concentration of GABA, the concentration of serotonin and the concentration of dopamine in the homogenate of the brains of mice after 30 days of treatment. Valproic acid stimulated an important diminish ($p < 0.001$) in the level of GABA, serotonin and dopamine in animals in the negative faction contrasted with animals in the normal control faction. Conversely, plant extract caused a notable expand ($p < 0.001$) in the concentration of GABA and serotonin at doses of 200 and 400 mg/kg and a significant growth ($p < 0.001$) in the concentration of dopamine at all doses tested in animals compared with animals in the valproate group. Risperidone also induced a serious ($p < 0.001$)

expand in these parameters in animals contrasted to animals in the valproate class (Figure 5).

Effect of hydroethanolic extract of *Crassocephalum crepidioides* on the histology of the mouse hippocampus.

Histological analysis in normal control animals showed a normal structure of the hippocampal parenchyma (Figures), with intact neurons in the different layers (GD, CA1, CA2 and CA3). In negative control animals compared with normal controls, a loss of neuronal tissue integrity was observed, marked by the presence of necrotic neurons with pycnotic (hyperchromatic) nuclei, neuronal loss, cytolysis and vacuolation. The batches receiving the extract at different doses and the batch receiving the reference substance showed a microstructure of the hippocampus close to that of normal (Figure 6).

DISCUSSION

Autism spectrum disorder (ASD) is a group of disorders characterized by varying degrees of impairment in social interaction and communication, as well as stereotyped behaviors and repetitive movements.²⁷ The valproic acid model is an animal model that better mimics the symptomatology of ASD in humans. Hence the general purpose of this work, which was to enhance the potential effects of the hydroethanolic extract (HEE) of *Crassocephalum crepidioides* (Cc) leaves on some autistic disorders induced in utero by valproic acid in mice. Alterations in social interaction are the main manifestations of autistic disorders and were assessed in this work in the three-chamber compartment test, the open arena test containing sawdust enabled us to assess stereotyped movements, and the elevated cross maze and dark and light box tests were used to assess anxiety as a comorbidity associated with autism.

In the sociability test, the important diminish ($p < 0.001$) in the time spent with the conspecific as well as the number of contacts with the conspecific was observed in animals in the negative control faction contrasted with those in the normal control class would mean a lack of affective contact in autistic mice. The diminish in time spent with a conspecific as well as the number of contacts indicates a lack of motivation to interact with its fellow animal.²⁸ This explains the ability of valproate in gestational administration to inhibit the initiation or maintenance of a social relationship. However, the improvement or reversal of these parameters in the class given hydroethanolic extract (HEE) of *Crassocephalum crepidioides* (Cc) at the different doses tested would justify the beneficial effects of this extract in improving social interaction disorders. The limbic system receives numerous projections from serotonergic neurons, and this system is responsible for regulating emotional expression and social behaviour.²⁹ The extract probably acts by stimulating the reward and motivation centres, leading to an increase in serotonin levels in the animals' brains.³⁰ This is in line with the work of ¹¹, who showed that *Bacopa monniera* wehst improves

behavioral disorders in autism induced by sodium valproate in rats. In order to confirm the nice effects of this extract on the symptomatic triad of autism, we also considered it useful to assess repetitive and/or stereotyped movements in the open arena test.

It was shown in this work that valproic acid induced an important expand ($p < 0.001$) in burrowing time and the number of grooming in animals in the negative control class contrasted to those in the normal control faction. This would be due to a failure of the autistic mice to vary their movements. Valproic acid is thought to have induced a mutation in the coding sequence of the serotonin transporter, which is directly associated with the presence of ASD, which is linked to stereotyped or repetitive behaviour.³¹ The amygdala is a structure of the limbic nervous system linked to the frontal lobes which, via the pre-motor cortex, participates in the selection of sequences necessary for the development of a movement, and the prefrontal cortex which adapts the movement according to the context.²⁹ Impairment of this system is thought to be the cause of the animals' inability to initiate new movements. CC EHE at the different doses tested resulted in a decreased considerably ($p < 0.001$) in burrowing time and the number of grooming in animals in the test factions opposed to animals in the negative control class.

Serotonin, dopamine and GABA are the neurumediators most involved in the regulation of motivation and emotion.³² The considerable decrease ($p < 0.001$) in the concentration of these three monoamines in animals in the negative control class opposed with those in the normal control group partly explains the impaired social interaction, repetitive movements due to lack of motivation and also the anxious behavior in autistic mice. In addition, brain serotonin levels appear to be reduced in people with autism.³³ The significant increase ($p < 0.001$) in these parameters in mice given Cc EHE at the different doses tested suggests that this extract acts via the serotonergic, dopaminergic and GABAergic systems to remedy some of the disorders and co-morbidities associated with autism. GABA increases chloride ion transport to the intracellular environment, inducing hyperpolarisation, making the cell insensitive to certain stimuli, thereby reducing excitability in the central nervous system.³⁴ The brain is an organ that is particularly vulnerable to the harmful effects of oxidative stress because of its strong energy activity, which is the source of high oxidative metabolism, its higher concentration of iron in the basal ganglia, which can catalyst the liberation of reactive oxygen species, and its high concentration of polyunsaturated fatty acids, which are targets of lipid peroxidation.³⁵ Oxidative stress has a significant role in the pathogenesis of brain lesions by causing direct damage to the tight junction proteins of the BBB.³⁶ MDA is the end product of lipid peroxidation and its important reduction ($p < 0.001$) in groups treated with Cc extract suggests that this extract could prevent lipid peroxidation. Flavonoids and tannins contained in this extract would have acted by reducing the concentration of MDA. The presence of the

OH class on the chemical structure of flavonoids and tannins enables them to trap radical species by giving up an electron.³⁷ The extract is also capable of inhibiting the transformation of hydrogen peroxide into hydroxyl radicals by reducing ferric iron (Fe^{3+}), an essential element for the production of hydroxyl radicals, into ferrous iron (Fe^{2+}) in vitro. This reducing power is thought to be due to the presence of phenolic compounds in the extract, in particular flavonoids and hydrolysable tannins.³⁸ SOD and catalase are both enzymatic antioxidants that protect the cell against damage from reactive oxygen species.³⁹ They play a key role in the detoxification of superoxide anion. In fact, SOD catalysis the breakdown of superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2), which is an unstable molecule that is highly toxic to the cell.⁴⁰ The action of SOD is thus coupled to that of catalase, which is responsible for eliminating H_2O_2 by converting it into H_2O and O_2 .⁴¹ The considerably increase ($p < 0.001$) in the activity of these two enzymes suggests that CC extract has an antioxidant effect. This suggests that CC EHE restores the pro- and antioxidant balance in brain tissue. The hippocampus is a brain structure strongly involved in social behaviour.⁴² Histology of the hippocampus showed that valproic acid caused alterations in the various structures and disorganisation of the cells. CC's EHE at different doses remedied this damage. This effect is thought to be due to the action of phenolic compounds such as flavonoids, which have various reparative properties in the brain, including the restoration of neuronal functions and neuronal regeneration.⁴³ This physical restoration is thought to be partly responsible for the recovery of certain behaviors observed, such as socialization and repetition.

This study has certain limitations: it is an animal model; glutamate was not assayed.

CONCLUSION

This study shows that the beneficial effects of hydroethanol extract of *Crassocephalum crepidioides* on the nervous system led to a general improvement in autism spectrum disorders induced *in utero* in mice by valproic acid. The effects observed on social interaction and stereotypic movements are thought to be due to the presence in this extract of secondary metabolites such as flavonoids, which have anti-oxidant and neurogenic properties.

ACKNOWLEDGEMENTS

The authors are grateful to the head of the Laboratory of the Department of Biological Sciences, Faculty of Sciences, for providing the facilities. The authors are also grateful to the head of the Laboratory of Animal Physiology, Department of Biology and Animal Physiology of the Faculty of Sciences, University of Yaounde, Cameroon, for providing facilities for the histological and chemical essays.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: Animals were handled according to the guidelines of the Cameroon Bioethics Committee (reg. no. FWA-IRB00001954). The protocol was approved by the ethics committee of the Faculty of Sciences of the University of Maroua (ref. no. 14/0261/Uma/D/FS/VD-RC). Each animal was tested in only one behavioural test and tests were made to minimize animal suffering

REFERENCES

1. Sala R, Amet L, Blagojevic-Stokic N, Shattock P and Whiteley P. Bridging the gap between physical health and autism spectrum disorder. *Neuropsychiatr Dis Treat.* 2020;16:1605-18.
2. CIM-10 Version. Disponible sur: Available at: <http://apps.who.int/classifications/icd10/browse/2008/fr/#/F84>. Accessed on 17th March 2023.
3. Fombonne E. Epidemiology of pervasive developmental disorders. *Pediatr Res.* 2009;65(6):591-8.
4. Amiet C, Gourfinkel-An I, Bouzamondo A, Tordjman S, Baulac M, Lechat P, et al. Epilepsy in autism is associated with intellectual disability and gender: evidence from a meta-analysis. *Biol Psychiatry.* 2008;64:577-82.
5. Poirier R, Jacquot S, Vaillend C, Souththiphong AA, Libbey M, Davis S, et al. Deletion of the Coffin-Lowry syndrome gene Rsk2 in mice is associated with impaired spatial learning and reduced control of exploratory behavior. *Behavior Genetics.* 2007;37(1):31-50.
6. Nicolini C, Fahnestock M. Le modèle de rongeur induit par l'acide valproïque de l'autisme. *Exp Neurol.* 2018;299 (Pt A):217-27.
7. Ospina MB, Krebs SJ, Clark B, Karkhaneh M, Hartling L, Tjosvold L. Behavioural and developmental interventions for autism spectrum disorder : a clinical systematic review. *PloS One.* 2008;3(11):e 3755.
8. McPheeters ML, Warren Z, Sathe N, Bruzek JL, Krishnaswami S, Jerome RN. A systematical review of medical treatments for children with autistic spectrum disorders. *Pédiatrie,* 2011;127(5):e1312-21.
9. Ernst E. Herb-drug interactions : potentially important but woefully under-researched. *European Journal of Clinical Pharmacology.* 2000;56(8):523-4.
10. Noordin MY, Kim WC, Shahid I, Maznah I. Phenolic Content and Antioxidant Activity of Hibiscus cannabinus L, Seed Extracts after Sequential Solvent Extraction. *Molecules.* 2012;17(2012):12612-21.
11. Sandhya T, Sowjanya J, Veeresh B. Bacopa monniera (L.) Wettst ameliorates behavioral alterations and oxidative markers in sodium valproate induced autism in rats. *Neurochemical Research.* 2012;37(5):1121-31.
12. Samson G, Geoffroy G, Ouedraogo RL, Timofte D, Foyet HS, Hilou A, Kiendrebéogo M. Anxiolytic and anti-depressant effect of Salvia spp. essential oil on rat

- model of Autism Spectrum Disorder. *Bulletin of Integrative Psychiatry.* 2020;84.
13. Adjatin A, Dansi A, Badoussi E, Loko YL, Dansi M, Gbaguidi F, et al. Pphytochemical sceening and toxicity studies of *Crassocephalum rubens* and *Crassocephalum crepidioides* consumed as vegetable in Benin. *Journal of Chemical and Pharmaceutical Research.* 2013;6:160–7.
 14. Sanjeev K. Activité anti inflammatoire, immunorégulateur et antigénotoxicité de l'extrait méthanolique de *Crassocephalum crepidioides*, 2018.
 15. Adedayo BC, Ganiyu O, Sunday I, Oyeleye II, Ejakpovi AA, Boligon ML. Blanching alters the phenolic constituents and in vitro Antioxidant and anticholinesterase properties of fireweed *Crassocephalum crepidioides*, 2015.
 16. Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power” The FRAP Assay. *Analytical Biochemistry.* 1996;239(1):70–6.
 17. Sun T, Tang J, Powers JR. Effect of Pectolytic Enzyme Preparations on the Phenolic Composition and Antioxidant Activity of Asparagus Juice. *Journal of Agricultural and Food Chemistry.* 2005;113:964-9.
 18. Ter Horst JP, van der Mark M, Kentrop J, Arp M, van der Veen R, De Kloet R, et al. Deletion of the forebrain mineralocorticoid receptor impairs social discrimination and decision-making in male, but not in female mice. *Frontiers in Behavioral Neuroscience,* 2014;8:26.
 19. Taiwe GS, Ngo Bum E, Dimo T, Weiss N, Moto FCO, Sidikki N, et al. Antidepressant, myorelaxant and anti-anxiety-like effects of *Nauclea latifolia* Smith (Rubiaceae) roots extract in murine models. *J Pharmacol,* 2010;6:364-71.
 20. Wilbur KN, Bernheim F, Shapiro OW. Determination of lipid peroxidation. *Archives of Biochemistry and Biophysics.* 1949;24:305-10.
 21. Mishra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *The journal of Biological Chemistry.* 1972;247:1972-3170.
 22. Shina AK. Calorimetric assay of catalase, *Analytical Biochemistry.* 1972;47(2):389-94.
 23. Robinson RG, Shoemaker WJ, Schlumpf MT, Bloom FE. Effect of experimental cerebral infarction in rat brain on catecholamine and behaviour. *Nature.* 1975;255(5506):332-4.
 24. Laprade N, Soghomonian JJ. Glutamate decarboxylase (GAD65) gene expression is increased by dopamine receptor agonists in a subpopulation of rat striatal neurons. *Mol Brain Research.* 1997;48(2):333-45.
 25. Lowe IP, Robins E, Eyermen GS. The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. *J Neurochem.* 1958;3(1):8-18.
 26. Cannet C. Artéfacts : les difficultés de la technique histologique de la circulation à la coupe. *Rev Fr Histotechnol,* 2006;19:71–84.
 27. American Psychiatric Association, Diagnostic and statistical manual of mental disorders (5th ed.), 2013 ; Arlington, VA: auteur; 2013.
 28. Kang J, Kim E. La suppression de la fonction des récepteurs NMDA chez les souris exposées avant la naissance à l'acide valproïque améliore les déficits sociaux et les comportements répétitifs. *Front Mol Neurosci.* 2015;;8:17.
 29. Anderson G M: Development and neurobiology, Part 4: Serotonin in autism, *Journal of the American Academy of Child & Adolescent Psychiatry.* 2002;41:1513-6.
 30. Murray F, Smith D W and Hutson P H: Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *Eur J Pharmacol,* 2008;583:115–27.
 31. Schneider T, Roman A, Basta-Kaim A, Kubera M, Budziszewska B, Schneider K and Przewlocki R : Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology,* 2008;33(6):728–40.
 32. Bourin M, Hascoët M. Implication of 5-HT2 receptor subtypes in the mechanism of action of the GABAergic compound etifoxine in the fourplate test in Swiss mice. *Behavioural Brain Research.* 2010;208:352-68.
 33. Makkonen I, Riikonen R, Kokki H, Airaksinen MM, Kuikka JT. Serotonin and dopamine transporter binding in children with autism determined by SPECT. *Dev Med Child Neurol.* 2008;50:593-7.
 34. Lopes F, Ganzer L, Borges A, Kochenborger L, Januário C, Faria S, et al. Effects of GABA ligands injected into the nucleus accumbens shell on fear/anxiety-like and feeding behaviors in food-deprived rats. *Pharm Biochem Behav,* 2012;101:41-8.
 35. Dringen R, Gutterer JM, Hirrlinger J. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. *Eur J Biochem.* 2000;267:4912-6.
 36. Élizabeth B. Stress oxydatif cérébrovasculaire et rupture de la barrière hématoencéphalique dans le syndrome de Wernicke-Korsakoff expérimental. Thèse de Doctorat, Université de Montréal, 2010.
 37. Boubekri C. Etude de l'activité antioxydante des polyphénols extraits de *Solanum melongena* par des techniques électrochimiques, Thèse Doctorat, Université de Biskra Algérie, 2014;56.
 38. Alioune F, Ndiaye A, Hounda F, Ndiefi FJ, Mbaye D, Mbacké DS et al. Phytochemical screening, polyphenol content and antioxidant studies of ethanol leaf extract of *Combretum aculeatum* Vent, *European Journal of Medicinal Plants.* 2015;10:1-7.
 39. Luca M, Luca A, Calandra C. The Role of Oxidative Damage in the Pathogenesis and Progression of Alzheimer's Disease and Vascular Dementia. *Oxid Med Cell Longev.* 2015;504-678.

40. Jie L, Wuliji O, Wei L, Zhi-Gang J, Hossein AG. Oxidative Stress and neurodegenerative Disorders. *Int J Mol Sci.* 2013;14(12):24438-75.
41. Garait B. Le stress oxydant induit par voie métabolique (régimes alimentaires) ou par voie gazeuse (hyperoxie) et effet de la GliSODin. (PhD thesis), Université Joseph Fourier, grenoble, 2006;219p.
42. Sodhi MS, Sanders-Bush E. Serotonin and brain development. *Int Rev Neurobiol.* 2004;59:111-74.
43. Vauzour, Rodriguez-Mateos A, Corova G, Oruna-Concha M, Spencer J. Polyphenols and human health: prevention of disease and mechanisms of action. *Nutrients.* 2010;2:1106-31.

Cite this article as: Folefack IA, Djiogue S, Beppe JG, Allah-Doum NG, Adjoffoin CN, Djikem RNT, et al. *Crassocephalum crepidioides* extracts reverses the effects of valproic acid in an in-utero mouse model of autistic disorders. *Int J Res Med Sci* 2025;13:1808-17.