



Research Article

Neuroprotective Effect of Ethanolic Extract of *Sida acuta* in Mercuric Chloride-Induced Brain Damage in Rats

O. Owoye*, S.O. Agboola

Department of Anatomy, College of Medicine, University of Ibadan.

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Abstract

Mercuric chloride has useful applications but exhibits toxicity which affects the central nervous system thus causing health hazards. Antioxidants reduce the oxidative damage associated with mercury intoxication. We tested the hypothesis that administration of antioxidant-containing ethanolic extract *Sida acuta* (EESA) should attenuate the effect of mercuric chloride (HgCl₂) in brain of rat. Twenty male rats were randomized into four groups (n=5): Group A: Control, distilled water; Group B: EESA (200 mg/kg bwt); Group C: HgCl₂ (4 mg/kg bwt); Group D: HgCl₂ (4 mg/kg bwt) + EESA (200 mg/kg bwt). All treatments lasted seven days and were given orally by gavage. All rats were euthanized on day 8 of the experiment after conducting behavioural tests. Biochemical parameters namely: malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) and brain tissue were examined with regard to histological and histomorphometric parameters. Mercuric chloride significantly ($p < 0.05$) elevated the level of MDA and reduced the level of GSH, activities of SOD and CAT significantly ($p < 0.05$) relative to control. It also neuronal degeneration and significantly ($p < 0.05$) reduced the densities of viable of the cerebral cortex, dentate gyrus, pyramidal neurons of cornu ammonis of hippocampus, and Purkinje neurons of the cerebellum when compared with control. In Group D rats, the altered parameters were reversed to near control values significantly ($p < 0.05$), histological alterations were improved relative to HgCl₂-treated group. Data demonstrated that HgCl₂ exposure induced a significant degeneration in the cerebral cortex, dentate gyrus, cornu ammonis and cerebellum of Wistar rats which concomitantly administration of EESA with HgCl₂ ameliorated.

Key Words: broom weed extract, brain damage, mercury chloride, neuroprotection

INTRODUCTION

Mercury has been described as the third most dangerous heavy metal whose toxicity causes serious risks to health through its unfavorable biochemical effects (Celikoglu *et al.*, 2015). It is released into the environment by a variety of human activities such as mining, industrial, residential heating systems and waste incinerators. Mercury occurs in three different forms: the metallic form, inorganic salts, and organic compounds (Xu *et al.*, 2012) and its toxicity will depend on the form of mercury, route of entry, and dosage. Human exposure to mercury could be through its vapour, mercury chloride, or methyl mercury compounds (Rao and Purohit, 2011). Large human population could be affected through consumption of fish that has previously consumed inorganic mercury present in water sediments as part of the aquatic food chain (Uma *et al.*, 2012). Mercury has found useful application in various areas. For example, methyl mercury is an organic mercury compound, and in the form of thimerosal has been used as a preservative in vaccines routinely given to children, including diphtheria-tetanus-pertussis (DTP), hepatitis B, and some Haemophilus influenzae type B (Guzzi *et al.*, 2012). Medical uses of mercury include: as components of disinfectants, sphygmomanometers, clinical thermometers, skin lightening cream, germicidal soaps, teething powders, and some medications (Clarkson, 2003).

In spite of its useful application in various forms, mercury exhibits diverse toxicities which include: neurotoxicity, nephrotoxicity, reproductive toxicity and gastrointestinal toxicity with ulceration and hemorrhage (Moraes-Silva *et al.*, 2014; Rao and Purohit, 2011; Uma *et al.*, 2012; Xu *et al.*, 2012). Mercury is a major neurotoxicant, primarily affecting brain tissue causing brain damage especially of the cerebellum and the cerebral cortex (Xu *et al.* (2012). Reports have shown that among others, one of the mechanisms proposed for inorganic mercury toxicity include the binding of mercuric ions to sulfhydryl groups resulting in decreased glutathione levels and depletion of thiols leading to an increase of reactive oxygen species (ROS), ultimately leading to oxidative stress and increased neurotoxicity (Rao and Purohit, 2011). Oxidative stress has been defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to molecular damage as well as damage of organs (Celikoglu *et al.*, 2015). However, antioxidants are known to ameliorate oxidative stress by inhibiting or scavenging the free radicals generated to prevent damage (Owoeye and Ojora, 2015).

Mercury was reported to be a major neurotoxicant (Xu *et al.*, 2012) but Mottay and Neergheen-Bhujun (2015) have demonstrated that bioactive plant constituents from traditional herbs can protect neurons against oxidative stress by restoring oxidative balance. The plant *Sida acuta*, belongs to the Malvaceae family, a readily available shrub in tropical areas in Africa, and commonly used in traditional healing. Known

*Author for Correspondence: +234-8033239973

E-mail: o.owoye@mail.ui.edu.ng; oowoye2001@yahoo.com

in English as ‘broom weed’, the Yoruba of southwest Nigeria calls it ‘Osekotu’ as it readily grows around habitations in farms and in bushes. The aerial parts (stem, stalk and leaves) of the plant are the most frequently used parts in treating asthma, fever, malaria, diarrhea (Karou *et al.*, 2003). Other reported uses are: as a contraceptive (Londonkar *et al.*, 2009) and antibacterial (Karou *et al.*, 2007). Of importance, is the presence of phenolic compounds and antioxidant activity via free radicals scavenging property demonstrated in the leaves of *Sida acuta* (Ekor *et al.*, 2010; Bahar *et al.*, 2013).

Although the cerebellum and cerebral cortex was reported to be the target of mercury intoxication (Xu *et al.* (2012), extensive damage to the hippocampal formation of rats has also been reported (Owoeye and Farombi, 2015). The hippocampus is critical for the formation of long-term contextual, spatial, and episodic memories (Alberini and Kandel, 2015). While the cerebellum regulates motor coordination, equilibrium, both saccadic and smooth eye movements and maintains muscle tone, the cerebral cortex is responsible for regulating cognition and primary sensory functions among other functions (Snell, 2006). The abundant lipid content and relative deficit in antioxidant systems compared to other tissues and high oxygen demand makes the brain susceptible and particularly vulnerable to damage by reactive oxygen species (ROS) than do most other organs (Ebokaiwe *et al.*, 2013). This susceptibility of the brain to oxidative damage in the presence of neurotoxicants may affect the histology and physiology of brain components in the absence of ameliorating factors like antioxidants.

Literature is scanty on the effect of ethanolic extract of *Sida acuta* (EESA) on HgCl₂-induced toxicity in the brain of Wistar rat and this study aimed at answering the question: “Can the ethanolic extract of *Sida acuta* modulate the effect of mercuric chloride on the brain of rat?”

MATERIALS AND METHODS

Experimental Design

The details of the experimental design are as shown in Table 1. All preparations were administered orally using a clean intra-gastric gavage for seven (7) days in each case. All rats were sacrificed on day 8 of the experiment. The study was conducted in the Department of Anatomy, and Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Table 1:
Animal grouping and Research Design.

Group	Treatment
I	Control, received rat chow and distilled water daily.
II	EESA (200 mg/kg body weight) daily
III	HgCl ₂ (4 mg/kg body weight) daily
IV	HgCl ₂ (4 mg/kg body weight) daily + EESA (200 mg/kg body weight) daily

EESA - Ethanolic extract of Sida acuta; HgCl₂ - Mercuric chloride. All preparations were administered orally using a clean intra-gastric gavage for seven days in each case and rats were sacrificed on day 8 of the experiment.

Behavioural tests

On the 8th day of the experiment, the rats were weighed and then subjected to (1) open field test and (2) forelimb grip strength test.

Open field test

This test assessed the general locomotive activity of rodents and was performed according to the method described by Olopade *et al.* (2012). Briefly, each rat was placed in an open field, a 72 by 72 cm square box with lines on the floor dividing it into 18 by 18 cm square that allowed the definition of central and peripheral parts. At the beginning of the session, each rat was placed in the centre of the arena and its activity was recorded for a duration of 5 minutes during which the following parameters were assessed: (a) horizontal movements, measured by the number of transitions/lines crossed, (b) vertical movement or rearing (the number of times the rat balances on its hind feet), (c) centre time (length of time spent in the centre square and (d) grooming. All these parameters were assessed and video-recorded by the same set of observers. At the end of each session, each rat was removed from the open field and the experimental chamber was thoroughly cleaned with 70% ethanol to eliminate olfactory bias and allowed to dry before introducing a fresh animal.

Forelimb grip strength test

This is a test of muscular strength in the forelimbs (Tamashiro *et al.*, 2000). In this test, the forepaws were placed on a horizontally suspended metal wire 2 mm in diameter, 1 m in length and placed 1 m above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded; a maximum of 2 minutes was given to each rat. Each animal was given two trials with a 30 min inter-trial rest interval. This test assessed muscle strength and balance.

Sample collection and histological preparation

On the completion of the behavioural tests on the 8th day of the experiment, each of the rats were anaesthetized with ketamine (100 mg/kg) intraperitoneally, followed by cervical dislocation. Each rat was decapitated at the cervico-medullary junction for uniformity; skulls were opened after which the brains were quickly extracted. Adopting the method of Igado *et al.* (2012), the right hemisphere of the each brain was preserved for histology. The cerebellum and brain of each rat were dissected out, rinsed and then preserved in 10% neutral buffered formalin and later processed for histology by paraffin embedment technique. The other half of the brain preserved for biochemical assays was rapidly rinsed, mopped with filter paper, weighed and kept in freshly prepared cold phosphate buffered solution (PBS) at pH=4 in the freezer till processed.

Histology

The cerebellum from each group was obtained and homologous sampling was assured by obtaining transverse sections of the right cerebellum from each specimen from the lateral zone portions of the cerebella hemisphere only for uniformity. Coronal sections of the right half of each brain were made to obtain samples of the frontal cerebral cortex and hippocampal tissue. The tissues were sectioned at 5-6 µm thickness using a Rotary Microtome (Leica RM2125 RTS), and then stained with Haematoxylin and Eosin according to the method of Bancroft & Gamble, (2008). After staining, the

slides were viewed with an Olympus CH (Japan) light microscope with 16x objective. The image capturing was performed with a Sony DSC-W 30 digital camera (Japan) and photomicrograph calibration was done with 'Image J' (Abramoff *et al.*, 2004).

Biochemical Assays

The left hemisphere of the brain samples was homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate centrifuged at 10,000 g for 15 minutes at 4 °C. The supernatant was collected for the estimation of the various biochemical bioassays. Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi *et al.* (2000) and expressed as micromoles of MDA per milligram protein. Protein concentration was determined by the method of Lowry *et al.* (1951). Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich (1972). Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow *et al.* (1974). Catalase (CAT) activity was determined using hydrogen peroxide as substrate according to the method of Clairborne (1995). Biochemical assays were conducted at the Drug Metabolism & Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Histomorphometry

Tissues from the rats used for quantification were studied using an Olympus CH (Japan) binocular microscope at ×40 magnification and photographed by a digital camera (Sony DSC-W 30 Cyber-shot). The densities of the frontal cerebral cortical neurons, granule neurons of dentate gyrus, pyramidal neurons of the cornu ammonis3 (CA3) and cornu ammonis 1 (CA1) of the hippocampus, and Purkinje neurons of cerebellum, were measured using a microscope with a graticule at different magnifications according to reported methods (Owoeye *et al.*, 2010). Briefly, the micrometer was calibrated using a stage micrometer slide with a customized 2 mm ruler engraved on the cover slip (Leitz, Wetzlar, Germany). This was done by using the eyepiece of an Olympus CH (Japan) binocular microscope at ×40 magnification. The radius of the eye piece at ×40 was calibrated with the graticule to be 0.19 mm, and the area of the view at ×40 magnifications was thus estimated as 0.11 mm². The densities of the cells on the histological slides were determined by counting the number of viable neurons (excluding degenerating neurons) observed within a given square area in a section (Sugihara *et al.*, 2000). For comparison, the densities of these viable neurons were also quantified using measured squares of the OpenOffice.org.Draw (Apache Open OfficeTM3). Measurements were made on each section from all experimental and control groups, and from slides of each group, ten observations were made at high-power fields. The average of the densities was calculated and compared by two investigators who independently quantified using the graticule and OpenOffice software methods.

Statistical analysis

All data were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to test for differences among all the groups. Dunnett's post hoc test using GraphPad Prism 6.0

software, San Diego, CA, USA. A p-value < 0.05 was considered statistically significant

Collection and Identification of Plant Materials

Fresh, healthy leaves of *Sida acuta* were harvested from bushes in the premises of University of Ibadan, Nigeria. The leaves were identified at the Botany Department, University of Ibadan, Nigeria and then authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria, by Mr L.T Soyewo with the Forest Herbarium Identification Number FHI 110161, where a voucher specimen was deposited.

Phytochemical Screening

Phytochemical analysis of the leaves of *Sida acuta* was carried out using simple standard chemical tests based on the protocol described by Harborne (1973).

Extraction procedures of ethanolic extract of *Sida acuta* (EESA).

The leaves were washed under running tap water to remove impurities and then air-dried at room temperature for seven days. The dried leaves were blended and 450 g of pulverized leaves was obtained. Extraction of plant constituent was by cold maceration in 99.9% ethanol (3 x 2.5 L) at room temperature, stirred daily over a period of 72 hours. The solvent-extract mixture was filtered with Whatmann filter paper number 2 and the extract solution obtained concentrated in a rotary vacuum evaporator until a solid residue was formed which weighed 21 g, giving a percentage yield of about 4.7%. The dry (solid) extract was termed ethanolic extract of *Sida acuta* (EESA).

Preparation, dosage and administration of EESA

From a stock solution of 1 g EESA/10mls of distilled water, the dissolved extract (EESA) was administered orally to the animals at a dose of 200 mg/kg based on the published method of Ekor *et al.* (2010).

Chemicals

Mercuric chloride powder (LobaChem PVT Ltd, Mumbai, 40005, India) was purchased from Julimark Enterprises, Yemetu, Ibadan, Nigeria. Ketamine was manufactured by Rotex Medica, Trittau, Germany. All other chemicals and reagents were purchased from British Drug Houses Poole, Dorset, UK. Phosphate Buffer Saline (PBS) at pH = 4.0 was prepared and stored in the refrigerator at 4°C.

Preparation, dosage and administration of mercuric chloride (HgCl₂)

From a stock solution of 100mg of HgCl₂ to 20mls of distilled water, HgCl₂ was administered to the animals at a dose of 4 mg/kg/daily for 7 days based on the method of Hussain *et al.* (1997) with the aid of an oral gavage.

Animal Management:

Healthy male Wistar rats (n=20), weighing between 105 g and 140 g were acclimatized for two weeks on rat chow and water ad libitum and thereafter randomized into experimental and control groups. The rat chows used were the product of Vital Feeds, Jos, Nigeria. The animals were housed in the Animal House of the College of Medicine, University of Ibadan in well-ventilated cages. All procedures on animal handling were in accordance with guidelines of the University of Ibadan

Ethical Committee which conformed to the ethical use of animals in research (PHS, 1996).

RESULTS

Physical observation: There was no mortality recorded in any of the groups throughout the duration of the experiment.

Phytochemical Analysis: The phytochemical evaluation of the leaves of *Sida acuta* showed the presence of flavonoids tannins, alkaloids, cardenolides, anthraquinones, steroids and terpenoids.

Biochemical parameters: Data presented in Table 2 indicated that HgCl₂ increased MDA and reduced GSH levels significantly (p<0.05) while also reducing the activity of the enzymes SOD and CAT relative to control. Concomitant treatment of HgCl₂ with EESA significantly reduced MDA level while elevating GSH level and the activities of SOD and CAT (p<0.05) when compared with the HgCl₂.

Behavioural results: The HgCl₂-treated rats were more active in horizontal transitions (LC) and grooming (GR) when compared to control which was significant (p<0.05) as shown in Table 3, however, it had no significant effect on the forelimb grip strength test (FLG), central square duration (CSD) and rearing (RE). Concomitant treatment of HgCl₂ with EESA recorded a significant (p<0.05) reduction in the LC, RE, and GR when compared with HgCl₂ only.

Histological parameters

Cerebral cortex: Mercuric chloride (HgCl₂) treatment caused degeneration of a sizeable portion of the frontal cortical neurons as shown by loss of basophilic staining of the nuclei as well as the presence of several vacuolar spaces indicating sites previously occupied by degenerated neurons (dn) indicated by arrowheads (Plate 1C) when compared with other

cortical neurons (cn) in Plates 1A and 1B. The impact of this neuronal loss could also be observed in the neuronal density displayed in Table 4. Concomitant treatment with EESA caused a reduction of the effect of HgCl₂ as shown in Plate 1D where the cortical neurons showed reduction of degenerated neurons though with pyknotic neurons which was also reflected in Table 4. The basophilic hue observed in Plates 1A and 1B was missing in the HgCl₂-treated groups which presented an eosinophilic hue in slides Plates 1C and 1D.

Dentate gyrus: The histological features of dentate gyrus showing molecular layer (ML), granule cell layer (GL) and the polymorphic layer (PL) are shown in Plates 2A and 2B. The effect of HgCl₂ is shown in Plate 2C as some granule neurons are pyknotic while some neurons are pale and denuded. Plate 2D shows some ameliorative effect of concomitant treatment with EESA with HgCl₂ when compared with Plate 2C, as more granule neurons are observed, a fact also observed in the neuronal density in Table 4. The basophilic hue observed in Plates 2A and 2B was missing in the HgCl₂-treated groups which presented an eosinophilic hue in slides Plates 2C and 2D.

Cornu ammonis3 (CA3) : The component of CA3 subfield viz: stratum oriens (SO), stratum pyramidalis (SP), and stratum radiatum (SR) presents normal histological features in Plates 3A and 3B, with the pyramidal neuron exhibiting open chromatin. In Plate 3C, HgCl₂ toxicity on the pyramidal neurons is shown by karyolysis of the neurons indicated by the arrow in addition to areas of vacuoles indicating sites previously occupied by degenerated pyramidal neurons and pyknotic survivors. The neuronal loss is displayed in Table 4. In Plate 3D, the effect of concomitant treatment of EESA and HgCl₂, the pyramidal neurons show fewer intercellular vacuolar areas compared with Plate 3D. The prominence of the radial features of stratum radiatum in the control group and the basophilic hue observed in Plates 3A and 3B are absent in the HgCl₂-treated Plates 3C and 3D

Table 2:

Effects of treatment with HgCl₂ and ethanolic extract of *Sida acuta* (EESA) on biochemical analysis of the brain of adult Wistar of male rats.

Group	GSH (µg/ml/mg protein)	LPO (µMDA/mg prot.)	SOD (units/mg protein)	CAT (Units/mL)
Control	6.66 ± 0.55	1.83 ± 0.20	1.12 ± 0.12	0.14 ± 0.01
EESA	6.31 ± 0.90	4.39± 0.58*	0.73 ± 0.36*	0.13 ± 0.02
HgCl ₂	4.79 ± 0.48*	7.02 ± 0.41*	0.79 ± 0.18*	0.09 ± 0.02*
HgCl ₂ +EESA	5.94 ± 0.67**	2.23 ± 0.59**	0.81 ± 0.16**	0.10 ± 0.01*

Values are presented as Mean ± standard deviation of five rats per group. LPO- Lipid peroxidation, SOD- Superoxide dismutase, CAT- Catalase, GSH- reduced Glutathione, EESA, 200 mg/kg ethanolic extract of *Sida acuta*; HgCl₂, 4 mg/kg. *P<0.05 versus Control; **P<0.05 versus HgCl₂.

Table 3:

Behavioural changes in rats treated with HgCl₂ and ethanolic extract of *Sida acuta* (EESA).

Treatment	CSD (s)	LC	RE	GR	FLG (s)
Control	1.20±0.45	47.33±6.50	13.25±3.30	18.33±4.50	5.50±1.73
EESA	1.00±0.00	40.00±6.08*	12.00±3.4	25.50±9.09*	3.20±0.84*
HgCl ₂	1.00±0.00	61.33±7.93*	11.25±3.30	42.00±7.09*	4.20±1.30
HgCl ₂ +EESA	1.20±0.45	45.75±5.28**	3.75±1.36**	34.25±6.19**	5.50±1.29

Data are expressed as mean ± standard deviation of five rats per group. Central Square Duration (CSD); Line Crossing (LC); Rearing (RE); Grooming (GR); Fore-Limb Grip (FLG). EESA, 200 mg/kg ethanolic extract of *Sida acuta*; HgCl₂, 4 mg/kg. *P<0.05 versus Control; **P<0.05 versus HgCl₂.

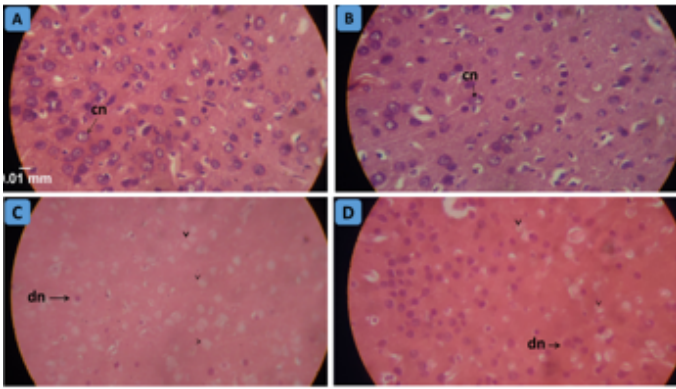


Plate 1: Representative stained sections of cerebral cortex of rats: (A) Control (B) EESA-treated (C) HgCl₂-treated shows degeneration of cortical neurons (D) HgCl₂+EESA-treated. EESA, 200 mg/kg ethanolic extract; HgCl₂, 4 mg/kg mercuric chloride; **cn**, cortical neurons; **dn**, degenerated neurons. H&E. Calibration bar = 0.01mm (10 μ m)

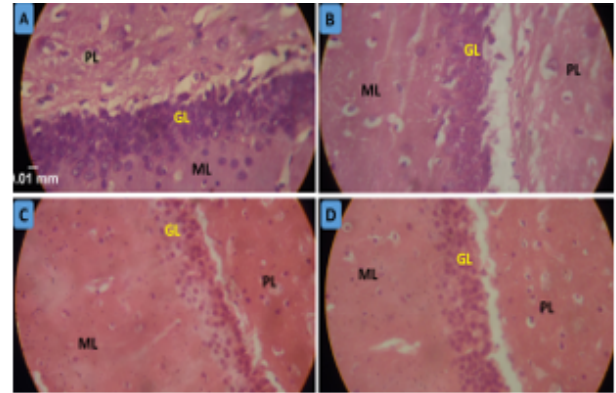


Plate 2: Representative stained sections of dentate gyrus of rats: (A) Control (B) EESA-treated (C) HgCl₂-treated group shows scattered pyknotic neurons in GL with vacuoles (D) EESA+ HgCl₂-treated show less pyknotic granule neurons. EESA, 200 mg/kg ethanolic extract; HgCl₂, 4 mg/kg mercuric chloride; ML, molecular layer; GL, granular cell layer; PL, polymorphic layer. H&E. Calibration bar = 0.01mm (10 μ m) for all figures

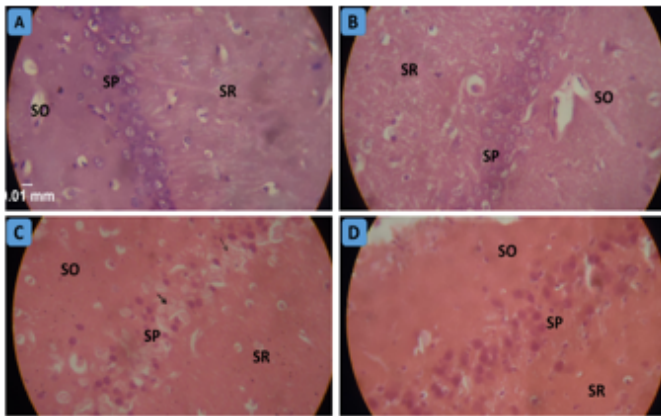


Plate 3: Representative stained sections of Cornu Ammonis3 of rats: (A) Control rats (B) EESA-treated (C) HgCl₂-treated showing scattered pyramidal neurons and vacuoles indicating sites of degenerated neurons (D) HgCl₂+EESA group show fewer intercellular vacuoles in the SP. EESA, 200 mg/kg ethanolic extract; HgCl₂, 4 g/kg mercuric chloride; SO, stratum oriens; SP, stratum pyramidalis; SR, stratum radiatum. H&E. Scale bar, 0.01mm (10 μ m) for all figures

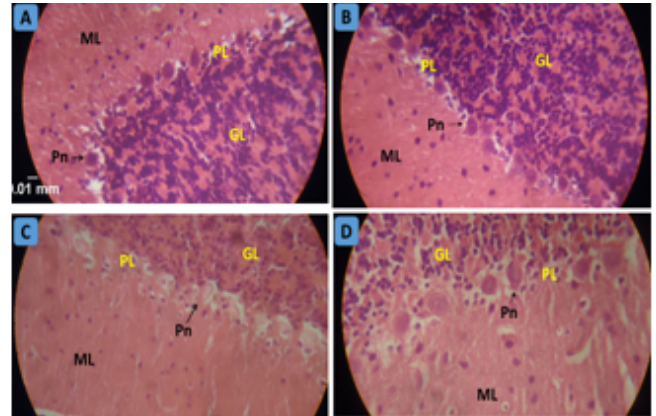


Plate 4: Representative stained sections of cerebellum of rats: (A) Control rats (B) EESA-treated (C) HgCl₂-treated (D) HgCl₂+EESA group. HgCl₂-treated show degenerated Purkinje neurons and complete eosinophilic stain. EESA, 200 mg/kg ethanolic extract; HgCl₂, 4 g/kg mercuric chloride; ML, molecular layer; PL, Purkinje cell layer; GL, granular layer; Pn, Purkinje neuron. H&E. Scale bar, 0.01mm (10 μ m) for all figures

Table 4: Effects of treatment with HgCl₂ and EESA on the densities of neurons of cerebral cortex and DG, CA3, and CA1 of hippocampal formation of rats.

Group	DG (/mm ²)	CA1(/mm ²)	CA3 (/mm ²)	CCN(/mm ²)
Control	16.9±0.13	4.7±0.02	6.5±0.06	4.4±0.35
EESA	14.9±0.09	4.1±0.03	5.9±0.05	3.4±0.19
HgCl ₂	4.2 ±0.01*	1.9 ±0.01*	2.1 ±0.01*	1.0 ±0.07*
HgCl ₂ + EESA	13.9 ±0.09**	3.1 ±0.01**	4.3 ±0.08**	2.4 ±0.15**

Data presented as Mean ± SEM of five animals per group. DG, dentate gyrus; CA1, cornu ammonis1; CA3, cornu ammonis3; CCN, cerebral cortical neurons; EESA, 200 mg/kg ethanolic extract of *Sida acuta*; HgCl₂, 4 mg/kg Mercuric chloride. *P<0.05 versus Control, **P<0.05 versus HgCl₂.

Cerebellum

In Plate 4A and 4B are shown the micro-anatomical layers of the adult rat cerebellum viz: granular (GL), molecular (ML), and Purkinje (PL). The Purkinje neurons in the PL in Plate 4C demonstrates the effect of HgCl₂ which includes loss of nuclear materials and cellular membranes and lack of basophilic staining when compared with Plates 4A and 4B. Observe in Plate 4D, the better preserved and basophilic staining of the Purkinje neurons as well as their cellular integrity when compared with the HgCl₂ group of Plate 4C.

Histomorphometry

Density of viable dentate granule, pyramidal and cerebral cortical neurons: Table 4 presents a summary showing that HgCl₂ significantly (p<0.05) reduced the densities of the viable neurons of the dentate gyrus, pyramidal CA1 and CA3

layers relative to the control group. Similarly, it is noted that HgCl₂ reduced the densities of the frontal cortical neurons significantly ($p < 0.05$). In varying degrees, EESA concomitant treatment with HgCl₂ significantly ($p < 0.05$) increased the densities of all the parameters when compared with the group HgCl₂ thus ameliorating the mercury effect.

DISCUSSION

In this present study, our data demonstrated the damage caused to brain tissue by administration of mercuric chloride (HgCl₂, 4 mg/kg) to rats for 7 days. We observed the ameliorating effect of ethanolic extract of *Sida acuta* leaves (EESA, 200 mg/kg) on the brain histological alterations, oxidative and behavioural changes.

The increased production of MDA in the brains of HgCl₂-treated rats, is indicative of damage to cellular membranes since an elevated MDA is a biomarker of oxidative stress in any system because this condition disrupts the physicochemical properties of the lipid bilayers of membrane, which leads to cellular dysfunction (Abolaji *et al.*, 2016), which in this case is lipid peroxidation (LPO) (Abdel, 2015). The elevated LPO, reduced GSH levels and reduction in the activities of SOD and CAT in HgCl₂-treated rats' further support a state of oxidative stress (Adedara *et al.*, 2015). The reduction in the activities of SOD and CAT may be due to deactivation of enzyme activity or possible structural alteration due to HgCl₂ exposure. The observed decrease in SOD activity and elevated LPO by *Sida acuta* alone suggested a possible deleterious effect of this extract at the investigated dose, the reason for which we cannot explain at present but requires our further investigation for clarification. However, our report of a significant lowering of the MDA level and elevation of the GSH by EESA (200 mg/kg) is in agreement with the findings of Ekor *et al.* (2010). This antioxidant activity may be due to presence of flavonoids as bioactive compounds in this extract (Karou *et al.*, 2007). Increased activities of SOD and CAT in the group treated concomitantly with both HgCl₂ and EESA suggest that the antioxidant activity exhibited by EESA was able to mitigate the event of the increased free radicals generated by HgCl₂ as reported (Ekor *et al.*, 2010; Adedara *et al.*, 2015).

The death of neurons of frontal cerebral cortex and the reduction in density in HgCl₂-treated rats shown by presence of degenerated neurons is in agreement with reported findings (Ferraro *et al.*, 2009; Owoeye and Farombi, 2015) that the cerebral cortex is often a target of mercury intoxication. This present results however, shows severe neuronal death not limited to pyknosis but extensive karyolysis of the neurons when compared with the earlier report of Owoeye and Farombi (2015). The difference may be explained by the fact that antioxidant was administered for 14 days before the exposure to HgCl₂ their study unlike the concomitant treatment of both extract and HgCl₂ in this present experiment. Our histological finding of unaltered cerebral neurons in the EESA-treated rats is in contrast to that of Eluwa *et al.* (2013) who reported that extract elicited hypertrophy of the cortical neurons.

The neuronal degeneration observed in the granule layer of the dentate gyrus (DG) of the rats' supports the toxicity of HgCl₂ and is in agreement with previous report it injured and elicited neuronal cell death in this subfield of the hippocampal formation (Owoeye and Farombi, 2015). The effect of HgCl₂ exposure on the pyramidal neurons of the cornu ammonis 3

(CA3) and the reduction in the density shows severe alteration of the histology due to its toxicity as many neurons exhibited cell death characteristics such as pyknosis and karyolysis with the cellular vacuoles (Stevens and Lowe, 2000).

The alteration observed in HgCl₂-treated cerebellum shows clearly that it damaged DNA according to the reports of Uma *et al.* (2012) and Bernhoft (2012). The complete loss of nuclear material, collapse of the Purkinje cells, and loss of basophilia is in agreement with earlier reports (Ibegbu *et al.*, 2014; Owoeye and Farombi, 2015). Being the focal neuron of the cerebellar cortex on which all afferent pathways ultimately converge on, the axons of Purkinje cells constitute the major exit from the cerebellar cortex. This explains why damage to Purkinje neurons affects movement, posture and balance (Snell, 2006). The increase in line crossing and forelimb strength of HgCl₂-treated rats was in contrast to the report of (Owoeye and Farombi, 2015) which reported a diminution of these parameters, a possible explanation may be the acute duration of the present experiment. All of these microscopic alterations observed in this experiment might be due to the greater vulnerability of the brain to oxidative damage due to its high lipid content, high oxygen demand and low level of endogenous antioxidant enzymes (Iriti *et al.*, 2010; Ebokaiwe *et al.*, 2013).

The implications of HgCl₂-induced neuronal death of cerebral cortical neurons is the reduction in effectiveness of the cognitive functions associated with the frontal cortex. A combination of granule neuronal death and CA3 pyramidal neuronal death will inevitably affect the trisynaptic pathway associated with the flow of hippocampal neural flow. The entorhinal cortex (EC) in particular, layer II neurons send their axons via the perforant path to the dentate gyrus (DG) and areas CA3 and CA2. Thus the excitatory synaptic input from the EC project on apical dendrites of DG granule cells, which give rise to mossy fibers. These cells in turn synapse on CA3 pyramidal neurons which via its glutamatergic Schaffer collaterals project onto ipsilateral CA1 pyramidal neurons, thereby completing the hippocampal trisynaptic circuit (Stepan *et al.*, 2015). The death of these neurons will inevitably lead to reduced quality in the acquisition and recall of episodic and spatial memories in such animal (Scharfman, 2007; Alberini and Kandel, 2016). Death of Purkinje neurons will inevitably affect cerebellar role in the coordination of voluntary skeletal muscle movement, posture and gait leading to such features as muscular hypotonia, intentional tremor, nystagmus (Ibegbu *et al.*, 2014; Snell, 2006).

However, previous reports (Hussain *et al.*, 1997; Augusti *et al.*, 2007; Rao and Purohit, 2011; Uma *et al.*, 2012) have demonstrated that the reported mechanism of mercury intoxication through oxidative damage can be mitigated by antioxidants. Hence the finding of ameliorative changes observed biochemically and histologically in all the groups concomitantly treated with EESA and HgCl₂ suggesting the mitigation or reduction of the above implications in the affected animals. This was made possible by the demonstrated antioxidant capacity of EESA (200 mg/kg) which reduced the level of LPO generated, enhanced the activities of the endogenous enzymes SOD and CAT and increased the level of GSH (Ekor *et al.*, 2010) all working in concert to mitigate HgCl₂ toxicity.

In conclusion, our data demonstrated that HgCl₂ exposure induced a significant degeneration in the cerebral cortex, dentate gyrus, cornu ammonis and cerebellum of Wistar rats.

However, EESA when concomitantly administered with HgCl₂ reduced these effects presumably via its antioxidant property. Although the data cannot be directly extrapolated to humans since pharmacokinetic parameters are different and have not been taken into consideration, it is appropriate that we counsel that precautions be taken to ensure careful use of dental amalgams, proper screening of drugs and vaccine preservatives, and education and protection for exposed workers.

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