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Monosodium Glutamate Toxicity: *Sida acuta* Leaf Extract Ameliorated Brain Histological Alterations, Biochemical and Haematological Changes in Wistar Rats

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ABSTRACT

The brain is reportedly sensitive to monosodium glutamate (MSG) toxicity via oxidative stress. *Sida acuta* leaf ethanolic extract (SALEE) possesses antioxidant activity which can mitigate this neurotoxicity. The present study investigated the possible protective effect of SALEE on MSG-induced toxicity in rats. Twenty-six female rats were randomized into four groups: I: control, feed and water only; II: 400 mg/kg daily SALEE; III: 4 g/kg MSG daily; IV: 400 mg/kg SALEE and 4 g/kg of MSG daily. All administration was oral and lasted 14 days. On 15th day, behavioural tests were done and thereafter, rats were euthanized with injection Ketamine. Blood and biochemical parameters were assessed and brain tissue was examined with regard to histological and histomorphometric parameters. Data indicated that MSG significantly ($p<0.05$) elevated MDA level, reduced GSH level and the activities of SOD and CAT, reduced PCV level, and neutrophil count. MSG also distorted the micro-anatomy of cerebellar Purkinje cells and pyramidal neurons of CA3. Co-treatment of SALEE with MSG significantly ($p<0.05$) reversed these changes back to near control values when compared with the MSG group. Our data support the fact that MSG may be detrimental to the brain but that oral co-administration of 400 mg/kg SALEE with 4 g/kg MSG may provide relative protection from MSG-induced oxidative impairment and the microscopic alterations of the rat brain.

Keywords; *Monosodium glutamate, Sida acuta ethanolic extract, neurotoxicity, oxidative stress, Purkinje neurons*

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INTRODUCTION

Monosodium glutamate (MSG), the sodium salt of glutamic acid is a natural constituent of many protein-rich food items such as meat, fish, cheese and some vegetables which is used worldwide as a flavor enhancer increasing the palatability of food and thus food intake (vonDiemen and Trindade, 2010; Hashem *et al*., 2012). In Nigeria, MSG is popularly used as a food additive and sold in most open market stalls and stores as "Ajinomoto" or "Vedan" or "White Maggi" (Eweka and Om'Iniabohs, 2007).

Despite its widespread use as food flavor, there are reports which indicate that MSG is toxic to humans and laboratory animals especially at high doses (Tawfik and Al-Badr, 2012). Its neurotoxicity which may lead to neuronal degeneration, is thought to be due to its well-known excitotoxic effect, since MSG can overexcite neuronal cells to the point of damage or death, resulting in brain injury mostly accompanied by oxidative stress (Eweka and Om'Iniabohs, 2007). Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system stimulating glutamate receptors and plays important roles in both physiological and pathological processes (Mattson, 2008). Excess glutamate in synaptic junctions in high doses of MSG may lead to overstimulation of glutamate receptors leading to injury to neurons or even neurodegeneration. Neuroprotection against glutamate-induced neurotoxicity is therefore a therapeutic strategy for preventing and treating both acute and chronic forms of neurodegeneration (Meldrum, 2002).

Oxidative stress associated with MSG administration in rats have been prevented or ameliorated by the use of antioxidant complexes like vitamin C, vitamin E, and Quercetin (Farombi and Onyema, 2006; Seiva *et al*., 2012). There is increasing interest in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients of plant products and their ability to treat various diseases. Sida acuta is a shrub belonging to the Malvaceaea family indigenous to tropical

areas. Known as 'broom weed', the Yoruba of southwest Nigeria calls it 'Ọsẹkotu' as it readily grows around habitations in farms and in bushes. It has been used in traditional medicine for treatment of various ailments like asthma, renal inflammation, colds, fever, headache, ulcers and worms but researchers have reported other activities of the plant such as: antimalarial and antibacterial agents (Karou *et al*., 2007); thrombolytic activity (Bahar *et al*., 2013); analgesic and antidepressant-like properties (Ibironke *et al*., 2014). Interestingly, the leaves have been reported to act as corrosion inhibitor for Aluminium-Copper-Magnesium alloy in acidic medium (Ayeni *et al*., 2014). Studies of Sida acuta leaves have demonstrated biological activity shown by the identification of alkaloids, principally cryptolepine (Karou *et al*., 2007); flavonoids and antioxidant activity of the ethanolic extract of the leaf has been demonstrated (Ekor *et al*., 2010; Bahar *et al*., 2013).

The brain is more vulnerable to excess reactive oxygen species than do most other organs and is therefore particularly vulnerable to oxidative stress which is normally prevented through the actions of antioxidants (Ebokaiwe, 2013). Neurotoxicants may affect the microanatomy of brain components thus altering its normal function. For example the effective function of the cerebellum in the regulation of motor coordination, equilibrium, both saccadic and smooth eye movements and maintenance of muscle tone; the cognitive functions of the cerebral cortex and the memory storage functions of the hippocampus may be disrupted (Snell, 2006). MSG-induced injury of these neural components may adversely alter the microanatomy and physiology of affected parts. Despite the awareness of the potential toxicity of MSG on the brain, it is used as a major taste enhancer in most eateries and cafeteria in Nigeria. However, information is scanty on the potential of Sida acuta leaf ethanolic extract (SALEE) to mitigate MSG-induced effect on the brain.

This study aimed to investigate the possible toxic effect of MSG, a natural constituent of many food items on the cerebral cortex, hippocampus and the cerebellar cortex of adult Wistar rat and to evaluate the possible protection of SALEE, a readily available and cheap vegetable with antioxidant property in preventing this toxicity.

MATERIALS AND METHODS

Plant: Fresh leaves of *Sida acuta* were obtained in Ibadan, Nigeria in August, 2014. Identification and authentication were done at the University of Ibadan Botany Department and Federal Research Institute of Nigeria (FRIN), Ibadan, Nigeria respectively. A specimen voucher FHI 110161 was deposited at the latter.

Chemicals: Chemicals and reagents were purchased from Sigma Chemical Co. USA. Ethanol and other materials were of the highest analytical grade. Phosphate Buffer Saline (PBS) at pH = 4.0 was prepared and stored in the refrigerator at 4°C.

Extraction procedures and preparation of *Sida acuta* leaf ethanolic extract (SALEE): 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 600g of pulverized leaves was obtained. Extraction of plant constituent was by cold maceration in ethanol (3 x 2.5 L) at room temperature over a period of 72 hours. The solvent-extract mixture was filtered and the extract solution obtained concentrated in a rotary vacuum evaporator until a solid residue was formed which weighed 27 g, giving a percentage yield of about 5%. The dry (solid) extract was termed *Sida acuta* leaf ethanolic extract (SALEE)

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).

MSG Preparation and Administration: Four hundred and fifty-four grams of 99.9% monosodium glutamate (MSG) with the brand name "Vedan Super seasoning", Batch number: VD-001 (Vedan Enterprise Corporation, Lagos, Nigeria) was purchased from Bodija market, Ibadan, Nigeria. Ten gram of the crystals was grinded (to increase surface area) and dissolved in 20 mL distilled water to give a concentration of 0.5 g/ml and 4 g/kg body weight was orally administered using a clean gavage to the experimental animals.

Animals Management: Healthy female Wistar rats numbering (n=26) weighing between 110g and 190g were obtained from the Animal House of the College of Medicine University of Ibadan. They were housed in plastic cages with wood shavings in a fly-proof, freely ventilated and naturally illuminated animal rooms at room temperature with a 12hr light/dark cycle. The animals were acclimatized for two weeks and then randomized into experimental and control groups. The animals were fed with standard mouse (Ladokun Feeds Nig. Ltd, Ibadan, Nigeria) and drinking water *ad libitum.* 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).

Experimental Design: Adult rats (n= 26) were grouped into four groups:
Group 1 (n= 5) - Control group were fed with feed and water only
Group 2 (n= 5) - 400 mg/kg (single dose daily) of SALEE for 14 days
Group 3 (n= 8) - 4 g/kg (single dose daily) of MSG for 14 days
Group 4 (n= 8) - 400 mg/kg (single dose daily) of SALEE and 4 g/kg of MSG (an hour after SALEE administration) for 14 days.

The dosage of 400 mg/kg of body weight of SALEE was based on the reported method of Ekor *et al*. (2010) while that of 4 g/kg of MSG was based on the method of Onyema *et al*. (2012). All administration was orally given using a clean intra-gastric gavage. All rats were sacrificed on day 15 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.

Behavioural tests: Twenty four hours after completion of the experiment, all animals were weighed and then subjected to behavioural assessment paradigm, namely: Forelimb grip test and open field test.

Forelimb grip test: A modification of the method of VanWijk *et al*. (2008) in which each rat was suspended with both forepaws on a horizontal steel wire (1 meter long, diameter 7 mm) was employed. Each rat was held in a vertical position when its front paws were placed in contact with the wire. When the rat grasped the wire, it was released, and the latency to fall was recorded with a stopwatch. Rats were randomly tested and each animal was given two trials with a 30 min inter-trial rest interval thus enabling muscle strength and balance to be assessed.

Open field test : The methods of Mohammad *et al*. (2010) and Ibironke and Fasanmade, (2015) were employed with slight modifications. The apparatus consisted of a square arena (56×56×20 cm) made of white wood with its floor divided by lines into 16 squares that allowed the definition of central and peripheral parts. At the beginning of the session, each rat was individually placed in the center of the arena and its activity recorded for 5 minutes using a video camera. The total locomotion (number of floor units entered with all four paws), rearing frequency (number of times the animal stood up on its hind limb or with the fore limbs against the wall of the observation box or free in the air) and grooming frequency (number of body cleaning with paws or picking of the body and pubis with mouth and face washing actions) within each 5 minutes interval were recorded. The crossing numbers were indicators of locomotor while the rearing numbers indicated vertical and exploratory activities. 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.

Sample collection and histological preparation ; On day 15 of the experiment, after completion of the behavioural tests, all animals in both control and experimental groups were weighed. Blood was collected via the retro-orbital venous sinus into heparinized bottles for haematological parameters. Rats were thereafter euthanized by ketamine (100 mg/kg) i.p. followed by cervical dislocation. Each rat was decapitated at the cervico-medullary junction for uniformity and the skulls opened after which the brains were quickly extracted and weighed. We adopted the method of Igado *et al*. (2012) wherein the right hemisphere, was preserved for histology and fixed in 10% neutral buffered saline for three days. 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) and then kept in the freezer till processed. The cerebellum and cerebrum of each animal were dissected and then preserved in 10% formalin and later processed for histology by paraffin wax embedding technique.

Determination of haematological values: Haemoglobin levels were measured by the cyanomethaemoglobin method using CE 404 colorimeter (Cecil Instrument). The haematocrit or packed cell volume (PCV) was measured by the microhaematocrit technique using Hawsksley microhaematocrit after centrifuging and spinning for 5 min at 12,000xg before reading with haematocrit reader. The RBC, WBC and platelet counts were done by haemocytometer method. MCV, MCH and MCHC were then calculated indirectly by using standard formulae. Differential count was done from prepared blood smear on a clean glass slide observed under light microscope.

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). Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow *et al*. (1974). Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich (1972). Catalase (CAT) activity was determined using hydrogen peroxide as substrate according to the method of Clairborne (1995).

Histology: The cerebellum from each animal was dissected out and homologous sampling was assured by obtaining transverse sections of the right cerebellar hemisphere from each specimen from the lateral zone portions of the cerebella hemisphere for uniformity. Coronal sections of the right half of each brain were made to obtain samples of the cerebral cortex and hippocampal tissue. The brain was sectioned at 5-6 µm thickness with a Rotary Microtome (Leica RM2125 RTS, Germany) and then stained with Haematoxylin and Eosin according to the method of Bancroft and Gamble (2008). The slides were observed with an Olympus CH (Japan) light microscope looking for possible neuronal damage or alterations of the histologic features of the cerebral cortex, hippocampus and cerebellum. Photomicrographs were acquired with a Sony DSC-W 30 Cyber-shot digital camera (Japan) while photomicrograph calibration was done with Image J (Abramoff *et al.*, 2004).

Histomorphomometry: 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 cortical neurons, pyramidal neurons of the cornu ammonis3 (CA3) of the hippocampus and Purkinje neurons, 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 x40 was calibrated with the graticule to be 0.19 mm, and the area of the view at x40 magnifications was thus estimated as 0.11 mm^2. The densities of the cells on the histological slides were determined by counting the number of viable neurons (excluding those with nuclear pyknosis) 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, 25 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 analyses

The quantitative variables were described by the mean and standard deviation. These variables were compared among the groups by the test of variance analysis (ANOVA) followed by Dunnett's post hoc test to identify statistically significantly different groups using GraphPad Prism 4.0 version software, San Diego, CA, USA. A five percent significative level was used.

RESULTS

General observation

No mortality was recorded in any of the groups throughout the period of the experiment.

Phytochemical Analysis

Phytochemical evaluation of the leaves of *Sidaacuta* revealed the presence of alkaloids, cardenolides, anthraquinones, tannins, phlobatannins, steroids, terpenoids, and flavonoids.

Weight changes

There was increase in body weight at the end of the 14-day experiment in all groups as shown in Table 1 with the exception of the MSG group whose rats had a reduction of their average weight on the day of sacrifice.

Table 1:
Table showing percentage differences in weight of animal in each group.

Group	Initial avg. (g)	Day 14 avg. (g)	Difference (g)	% Difference
Control	114.0 ±13.42	131.0 ± 17.46	17.0 ±1 2.04	19.090
SALEE	107.0 ±7.58	119.0 ± 4.18	12.0 ± 4.472	11.200
MSG	176.0 ±19.49	170.0 ± 18.71	-6.0 ± 8.94	-0.389
MSG +SALEE	126.0 ±8.94	136.0 ± 4.18	10 ± 6.12	3.158

Data are expressed as Mean ± S.D of five animals per group. SALEE, 400 mg/kg *Sida acuta* leaf ethanolic extract; MSG, 4 g/kg monosodium glutamate; avg, average.

Haematological parameters

Erythrocytic values: The packed red cell volume (PCV) was elevated significantly ($p<0.05$) by treatment with SALEE, while MSG treatment significantly reduced the PCV when compared with control. Co-treatment of MSG with SALEE increased the PCV significantly ($p<0.05$) than MSG group according to Table 2. Other red blood cell parameters were not significantly altered.

Table 2:
Effects of SALEE and MSG on the Erythrocyte indices of adult Wistar rats

Parameters	Control	SALEE	MSG	SALEE +MSG
PCV (%)	45.60 ±2.70	49.20 ±4.21*	41.20 ±1.30*	47.20 ±4.15**
HB (g/dL)	15.40 ±0.88	16.62 ±1.17	16.12 ±1.42	16.76 ±1.05
RBC ($x10^6$/µL)	7.57 ±0.43	8.20 ±2.65	6.87 ±0.74	8.38 ±0.58
MCH (pg)	20.34 ±0.84	20.26 ±1.10	23.46 ±2.84	20.0 ±1.00
MCV (fL)	60.24 ±0.82	60.0 ±1.10	59.97 ±2.83	56.3.0 ±1.00
MCHC (g/dL)	33.77 ±0.84	33.18 ±1.10	39.12 ±2.84	35.51 ±1.00

*Values are presented as Mean ± standard deviation of five animals per group. PCV- Packed Cell Volume; Hb-Haemoglobin; RBC- Red Blood Cell Count; MCH, Mean Corpuscular Haemoglobin; MCV- Mean Corpuscular Volume; MCHC- Mean Corpuscular Haemoglobin Count. SALEE, 400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate.*p<0.05 versus Control; p<0.05 versus MSG.*

Leukocytic values: Although the total white blood cell count (TWBC) was not significantly altered, MSG treatment reduced significantly ($p<0.05$) both neutrophils and monocytes when compared with control. Co-treatment of MSG with SALEE however, increased the neutrophils significantly ($p<0.05$) when compared with MSG group as shown in Table 3.

Biochemical parameters

Data presented in Table 4 indicated that MSG increased MDA and reduced GSH levels significantly ($p<0.05$) while also reducing the activity of the enzymes SOD and CAT relative to control. Co-treatment of MSG with SALEE significantly reduced MDA level while elevating GSH level and the activities of SOD and CAT ($p<0.05$) when compared with the MSG group.

Behavioural tests

Locomotion frequency: Administration of MSG increased the locomotion frequency significantly ($p<0.05$) than in the control, while SALEE reduced it when compared with the control. However, concomitant treatment of MSG with

SALEE significantly reduced the locomotion frequency ($p<0.05$) than that of the MSG group (Table 5).

Rearing frequency: The rearing frequency was significantly ($p<0.05$) increased by the concomitant administration of MSG with SALEE than in the MSG group as shown in Table 5.

Table 3: Effects of SALEE and MSG on the leukocyte indices of adult Wistar rats.

Group	TWBC ($x10^3$/ µL)	LYMP ($x10^3$/ µL)	NEUT ($x10^3$/ µL)	MONO ($x10^3$/ µL)
Control	6.70± 1.45	62.00± 4.36	35.25±2.22	2.20± 0.84
SALEE	7.00± 3.81	57.80± 5.40	30.25±2.75	1.40± 0.55
MSG	6.14± 3.32	66.80± 7.92	25.00±4.00*	1.80± 0.45*
SALEE+ MSG	6.66± 3.16	63.40± 7.67	37.33±3.79**	1.60± 0.89

*Values are presented as Mean ± standard deviation of five rats per group. TWBC- Total White blood count, LYMP- Lymphocyte; NEUT- Neutrophils; MONO, Monocytes. SALEE, 400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate.*p<0.05 versus Control; p<0.05 versus MSG.*

Table 4: Effects of SALEE and MSG on biochemical analysis of the brain of adult Wistar rats.

Group	LPO (MDA $x10^{-4}$/mg prot.)	SOD (units/ mg protein)	CAT (Units/ mL)	GSH (µg/ml/ mg protein)
Control	1.9 ±0.14	1.045 ±0.11	0.1176 ±0.27	6.658 ±0.346
SALEE	4.6 ±0.34*	0.783 ±0.16	0.1163 ±0.30	6.248 ±1.044
MSG	6.6 ±0.92*	0.316 ±0.18*	0.0982 ±0.01*	4.200 ±0.297*
SALEE +MSG	5.3 ±0.92**	0.547 ±0.12*	0.1115 ±0.02*	6.235 ±1.058**

*Values are presented as Mean ± standard deviation of five rats per group. LPO- Lipid peroxidase, SOD- Superoxide dismutase, CAT- Catalase,GSH- reduced Glutathione, SALEE-400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate. *p<0.05 versus Control; p<0.05 versus MSG.*

Grooming frequency: Treatment with MSG and SALEE individually increased significantly ($p<0.05$) the grooming frequency of rats when compared with the control group. However, co-treatment of both MSG and SALEE reduced this frequency significantly ($p<0.05$) than the value of the MSG group as shown in Table 5.

Fore-Limb Grip Strength: There were no significant changes in the fore-limb grip strength in the various treatment groups as indicated in Table 5.

Table 5:
Behavioural responses of rats treated with SALEE and MSG

Group	LC	RE	GR	FLG
Control	47.33±6.50	13.25±3.3	18.33±8.5(	5.50±1.7
SALEE	40.00±6.08'	12.00±8.4	45.50±15.0	3.20±0.8
MSG	61.33±17.9:	11.25±3.3	42.00±17.0	4.67±2.0
SALEE+MS	45.75±15.2(	13.75±2.3	34.25±6.19	5.52±1.4

Data are expressed as Mean ± S.D of five animals. LC- Line Crossing, RE- Rearing, GR- Grooming, FLG- Fore-Limb Grip strength. SALEE, 400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg monosodium glutamate.

Histological parameters

Cerebral cortex: Observations in Plate 1 shows normal cortical neurons having nuclei with dispersed chromatin in Plates 1A, 1B and 1D, whereas in the MSG, the neurons exhibit condensed neurons in Plate 1C.

Cornu Ammonis3 (CA3): The layers of the CA3 subfield of the hippocampal formation showed normal histological features of the stratum oriens, stratum pyramidalis and stratum radiatum with normal cytoarchitecture as shown in Plates 2A, 2B, and 2D. In Plate 2C, MSG toxicity on the pyramidal neurons was exhibited by pyknotic neurons and disruption of the normal layers of the neurons. Plate 2D showed a reduction in the neurodegeneration observed in the MSG group as evidenced by increase in neurons with dispersed chromatin.

Cerebellum: The microscopic anatomy of the adult rat cerebellum, namely: molecular, Purkinje and granular layers are observed in Plates 3A, 3B, and 3D. MSG's effect on the Purkinje cells of the cerebellum is shown by collapsed and eosinophilic staining Purkinje neurons lacking basophilia (Plate 3C) relative to the control. Observe in Plate 3D, that Purkinje cells features are comparable with those of control and SALEE groups.

Histomorphometry

Thickness of cerebellar layers: All the three cerebellar layers were quantitatively reduced by MSG, though this was significant only in the molecular layer. However, concomitant treatment of MSG with SALEE reversed these alterations significantly ($p<0.05$) as shown in Table 6

Density of viable cerebellar neurons: Viable stellate cells in molecular layer, Purkinje neurons in the Purkinje layer and granule cells in the granular layer were counted. MSG significantly reduced the number of viable cerebellar neurons in the three layers ($p<0.05$) relative to the control. As shown in Table 7, SALEE+MSG treatment increased the viable neurons significantly than in the MSG group ($p<0.05$).

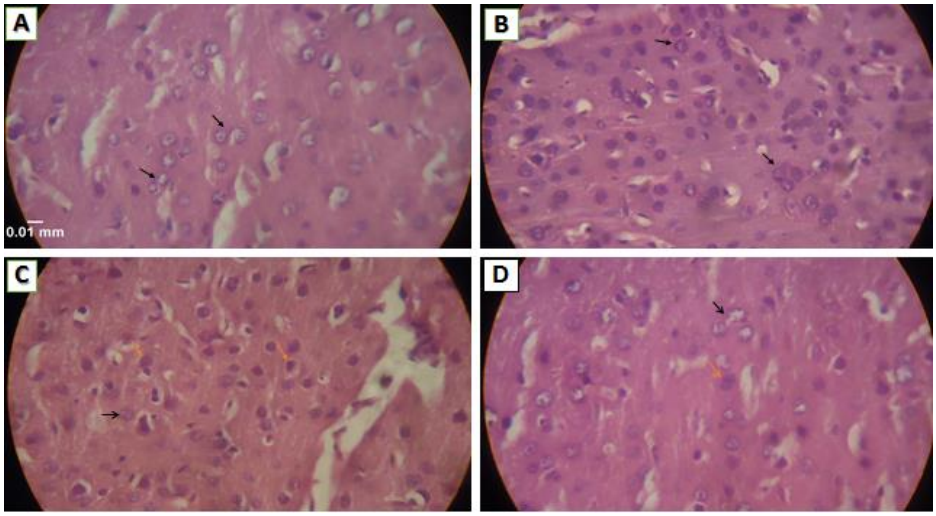

Plate 1:
Representative stained sections of cerebral cortex of rats: (*A) Control rats (B) SALEE-treated (C) MSG-treated (D) MSG+SALEE group. MSG-treated group show degenerated pyknotic neurons (orange arrows), normal cerebral cortical neurons show open faced nuclei (black arrows). SALEE, 400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate. H&E. Scale bar, 0.01mm (10 µm) for all figures*

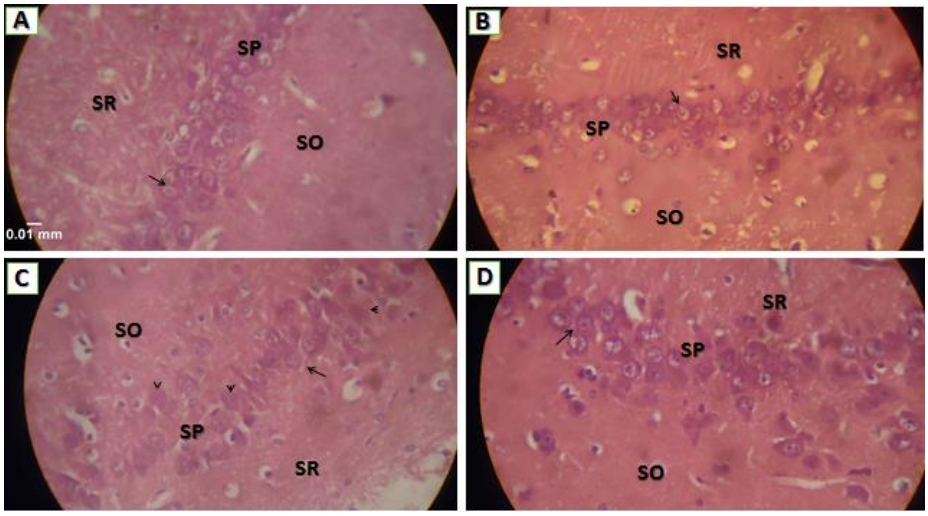

Plate 2:
Representative stained sections of Cornu Ammonis3 of rats: *(A) Control rats (B) SALEE-treated (C) MSG-treated (D) MSG+SALEE group. MSG-treated show degenerated neurons (arrowheads) and a distortion of the layered structure observed in the control group. Normal pyramidal neurons show open faced nuclei (arrows). SALEE, 400 mg/kg Sidaacutaleaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate; SO, stratum oriens layer; SP, stratum pyramidalis; SR, stratum radiatum. H&E. Scale bar, 0.01mm (10 µm) for all figures.*

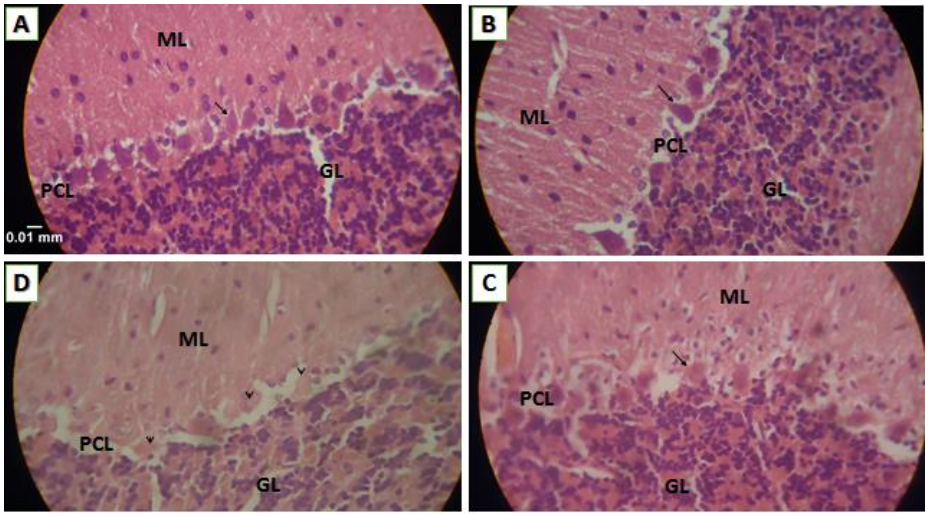

Plate 3:
Representative stained sections of cerebellum of rats: *(A) Control rats (B) SALEE-treated (C) MSG-treated (D) MSG+SALEE group. MSG-treated show non-viable degenerated Purkinje neurons (arrowheads) that have undergone karolysis. SALEE, 400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate; ML, molecular layer; PCL, Purkinje cell layer; GL, granular layer. H&E. Scale bar, 0.01mm (10 µm) for all figures*

DISCUSSION

In the present study, we investigated the ability of MSG to induce toxicity in rat brain. We also tested the possibility of SALEE to modulate the effect of MSG-induced toxicity in rat brain and other parameters. Our results demonstrated that SALEE could protect brain damage in MSG-treated rats. SALEE also ameliorated MSG-induced alterations in the haematological, biochemical and behavioural parameters.

Data obtained showed that MSG caused a significant weight reduction at the end of the experiment. Literature presents conflicting reports concerning the effect of MSG on weight changes in rats despite using the same or similar dosage. While our report is in agreement with those who reported similar findings of weight reduction by MSG treatment (vonDiemen and Trimidade, 2010; Lima *et al*., 2013), it is in conflict with those who reported an increase in weight leading to obesity (Ashry *et al*., 2012; Seiva *et al*., 2012), and those who observed no weight changes even after administration of the same 4 g/kg body weight of MSG to rats (Onyeama *et al*., 2012). The differences in results may be due to factors related to other rat parameters and environmental factors associated with the experimental procedures or differences in route of administration.

Table 6:
Effects of SALEE and MSG on the thickness of cerebellar layers.

Group	GL (µm)	PL (µm)	ML (µm)
Control	131.1±0.2	23.3±0.1	178.8±0.9
SALEE	122.5±0.4	22.9±0.6	173.3±1.1
MSG	97.1±1.6	21.6±0.2	131.6±0.3*
SALEE+MSG	130.4±2.2**	23.7±0.9	177.9±0.6**

*Values are presented as Mean ± standard deviation of five rats per group. GL- granular layer of cerebellum, PL- Purkinje layer, ML- Molecular layer. SALEE- 400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate.*p<0.05 versus Control; **p<0.05 versus MSG.*

Table 7:
Effects of SALEE and MSG on the viable neuronal cell count of cerebellum of Wistar rats.

Group	GL (no/nm^2)	PL (no/nm^2)	ML (no/nm^2)
Control	295.73±0.18	6.03±0.13	33.04±0.9
SALEE	265.38±0.37	6.68±0.55	37.0±1.01
MSG	196.20±11.2*	2.0±0.08*	29.2±0.28*
SALEE +MSG	308.11±8.06**	5.17±0.06**	48.0±0.62**

*Values are presented as Mean ± standard deviation offive rats per group. GL- granular layer of cerebellum, PL- Purkinje layer, ML- Molecular layer. SALEE-400 mg/kg Sida acuta leaf ethanolic extract; MSG, 4 g/kg Monosodium glutamate.*p<0.05 versus Control; **p<0.05 versus MSG.*

Furthermore, this study assessed the protective effect of SALEE on haematological parameters by measuring both red and white blood cells. Administration of MSG significantly depressed PCV and neutrophils in agreement with the findings of Ashaolu *et al*. (2011). However, SALEE significantly elevated the PCV and neutrophils in agreement with the report of Ekor *et al*. (2010) who made similar observation using 400 mg/kg body weight of ethanolic extract of Sida acuta leaf in alloxan diabetic rats. The restoration to near normal of PCV and neutrophils of rats co-treated with SALEE lend credence to its potential to obviate the noxious effect of MSG on these blood parameters.

A state of MSG-induced oxidative stress in the brain of rats was evidenced by the significant increase in MDA levels, decrease in GSH levels and reduction in the activities of SOD and CAT in MSG-treated rats in this present study which agrees published reports (Soliman, 2012; Farombi *et al*., 2003). Lipid peroxidation as shown by increase in MDA level is a major indicator of oxidative damage initiated by reactive oxygen species (ROS) resulting from MSG treatment (Onyema and Farombi, 2006). Reduction in the activities of SOD and CAT suggest inhibition arising from probable structural changes and loss of enzyme activity due to MSG exposure. The significant lowering of the MDA level in rats co-treated with SALEE group suggested a reduction in the extent of LPO implying that SALEE attenuated oxidative stress induced by MSG in this study. This is also supported by the significant reversing of the GSH depletion in the MSG-treated group which agreed with the report of Ekor *et al*. (2010) in a similar study. This might have been due to presence of flavonoids as bioactive compounds in this extract in agreement with the report of Karou *et al*. (2007). Increases observed in SOD and CAT in the co-treated groups suggest that adaptive responses was activated to mitigate the event of the increased free radical generation by MSG as reported by Adedara *et al*. (2015).The increase in antioxidant enzymes and GSH levels observed might have led to scavenging of the generated free radicals thus preventing oxidative stress in the rats co-treated with both SALEE and MSG. Since antioxidants are known to ameliorate the effect of oxidative stress, our study has demonstrated the ability of antioxidant property of SALEE in protecting the brain against MSG intoxication.

The altered microscopic observations in the brain sections confirmed the neurotoxicity of MSG group in agreement Eweka and Om'Iniabohs (2007) and Hashem *et al*. (2012). This was most evident in the CA3 and cerebellar cortex where pyramidal and Purkinje neurons respectively were disrupted and degenerated. The neuronal degeneration observed in the CA3 is in agreement with the reported MSG-induced hippocampal lesions (Zhang *et al*., 2012). Also, the lesion we report in the Purkinje neurons of the cerebellum in MSG-treated rats is similar to the findings of Hashem *et al*. (2012) who observed histological anomalies like pyknotic nuclei in Purkinje cells of MSG-treated brain. The neuronal damage in the Purkinje cells which were devoid of basophilic stain in MSG-treated rats may be due to damage caused by lipid peroxidation leading to enzyme inactivation, DNA damage and cell death due to oxidative stress (Schubert and Piaseki, 2001; Eweka and Om'Iniabohs, 2007). Our observation of normal cerebral neurons in the SALEE rats is in contrast to that of Eluwa *et al*. (2013) who reported that SALEE caused hypertrophy of the cortical neurons. The sustained high concentrations of MSG in the body, maintains a high concentration of glutamate, an excitatory amino acid, in the synaptic cleft region thus resulting in excessive glutamate receptor activation with persistent depolarization producing metabolic and functional exhaustion of the affected neurons and hence led to neuronal necrosis (Mattson 2008). According

to Bojanic *et al*. (2004), MSG can act as an ''excitotoxin'' implying that it can overstimulate neurons to the point of cellular damage or cellular death. This overstimulation is what the overall behavioural tests presents as that of overactive animals in the MSG group in both locomotion and grooming frequencies results.

These micro-anatomical alterations observed imply that the functions of the CA3 relative to memory might be hampered due to death of pyramidal neurons which is important in receiving impulses from the mossy fibres from granule neurons of the dentate gyrus of hippocampal formation (Snell, 2006). Similarly, the histological alterations caused by neuronal death of pyramidal cells of CA3 might disrupt the smooth flow of neural information coming from the entorhinal cortex via the granule cell neurons of dentate gyrus to CA3 via mossy fibres. Subsequent projection of impulses from CA3 via the Schaffer's fibres to CA1 may be affected thus altering the flow of neural information implying that memory and other hippocampal functions might potentially be affected in such rats (Scharfman, 2007). The MSG-induced Purkinje cell damage may cause cerebellar injury affecting movement and posture (Afifi and Bergman, 2005). Purkinje cells are the principal output neurons of the cerebellum (Snell, 2006) hence its important role in maintenance of balance and posture modulation. However, after co-administration of SALEE, these neurotoxic effects observed seem to have been counteracted by the antioxidant in SALEE which significantly ameliorated the MSG-induced cerebellar, hippocampal and cerebral cortical damage most likely through its antioxidant activity.

In conclusion, our findings support the fact that MSG despite its flavoring functions may be detrimental to the brain. We demonstrated that oral co-administration of 400 mg/kg SALEE with 4 g/kg MSG offered relative protection from MSG-induced oxidative impairment and the microscopic alterations of the cerebral cortical neurons, pyramidal neurons of the CA3 and cerebellar Purkinje neurons as demonstrated in the improvement of histological features observed in the treated brains of rat. Our data suggest that SALEE may be useful as a protective substance for further studies in the prevention of MSG-induced intoxication and to corroborate these findings.

Competing Interests

Authors declare that they have no competing interests.

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