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Neuroprotective potential of *Aframomum melegueta* extracts on brain of monosodium glutamate-treated wistar albino rats

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The study evaluated the biochemical effects of methanolic seed extract of *Aframomum melegueta* (MEAM) on biochemical parameters of the brain of rats treated with monosodium glutamate (MSG) with a view to considering the possibility of using the plant as a remedy for the management of neurotoxic disorders. MEAM was prepared according to standard methods, phytochemically screened and followed by evaluation of its antioxidant and anti-inflammatory potentials as well as its biological activities. Phytochemical screening of the methanolic extract (ME) revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. Administration of MSG (2 g/kg bwt) caused alterations in the levels of glutathione, nitric oxide, Vitamin C and E, as well as in the reduction of activities of enzymatic antioxidants. There were increases in the activities of brain marker enzymes, protein and peroxidation levels. Histopathological observations of brain sections revealed that MEAM protected the brain from glutamate-induced neurotoxicity. Conclusively, extract of *A. melegueta* elicited appreciable and potent neuroprotective potentials against neuro-degeneration caused by MSG oral administration, in a concentration dependent manner.

Key words: Neurotoxicity, *Aframomum melegueta*, methanolic extract, monosodium glutamate, antioxidant enzymes, neurotransmitters, brain biomarkers.

INTRODUCTION

Monosodium glutamate (MSG), the sodium salt of the non-essential amino acid glutamic acid is commonly consumed as a flavour enhancer. Though, there is still a lot of controversy on the neurotoxic effects of MSG, studies over 30 years revealed that scientists have used MSG to induce neurodegeneration *in vivo* and *in vitro* (Kubo and Kohira, 1993; Zhou, 2009). Monosodium glutamate (MSG) is a natural constituent of many protein-

rich food items, though it has been reported to elicit neurotoxic effects due to its deleterious effects on the cerebellum of wistar albino rats at higher concentrations. It affects the functions of the brain leading to tremor, unstable and uncoordinated movement and ataxia most probably through oxidative stress (Schubert and Piasecki, 2001; Eweka and Om'Iniabohs, 2007). The neurotoxic effect of MSG according to Mattson (2008) reported

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that high concentrations of MSG results in excessive glutamate receptor activation with persistent depolarization thereby producing metabolic and functional exhaustion of the affected neurons and hence neural necrosis.

In spite of its ubiquitous role as a neurotransmitter, glutamate is highly toxic to neurons, a phenomenon 'excitotoxicity' (Choi, 1998). Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by ROS (Murphy et al., 1990; Choi, 1998). Oxidative glutamate toxicity has been observed in primary neuronal cell cultures (Murphy et al., 1990; Oka et al., 1993) and tissue slices and has been studied recently in the immortalized mouse hippocampal cell line, HT22 (Lipton and Rosenberg, 1994; Sagara et al., 1998). Thus, neuroprotection against glutamate-induced neurotoxicity has been therapeutic strategy for preventing and/or treating both acute and chronic forms of neurodegeneration (Meldrum, 1998).

Aframomum melegueta is a species in the ginger family, Zingiberaceae. It is commonly known as grains of paradise (English), *Ataare* (Yoruba), *ósè ójì* (Igbo), Malagueta (Spanish) and Graines de paradis (French) (Noumi and Fozi, 2003). *A. melegueta* is a popular medicinal plant that is rich in phytochemicals that act in different ways to bring about human health benefits. Its oral ingestion increases whole body energy expenditure through the activation of brown adipose tissues (Sugita et al., 2013). Lee and Surh (1998) noted that *A. melegueta* vanilloids, 6-gingerol and 6-paradol, is capable of inducing apoptosis. It has been shown to exhibit anti-inflammatory activity, which is in part due to the inhibition of COX-2 enzyme activity and expression of pro-inflammatory genes (Nebojsa et al., 2014), reduces the frequency of abdominal constrictions induced by acetic acid in mice (Umukoro and Ashorobi, 2001), moderately inhibit acetylcholinesterase, α -amylase and α -glucosidase (Adefegha and Oboh, 2012 a, b), and exhibited a dose-dependent decrease in blood glucose (Ilic et al., 2010).

This study seeks to investigate both protective and ameliorative effects of methanolic extract of *A. melegueta* on the selected biochemical parameters of the brain of rats treated with MSG with a view to providing additional information on the phytochemicals and contributing to the on-going search for natural antioxidants as alternatives to synthetic ones.

MATERIALS AND METHODS

Collection and identification of plant material

Dried fruits of *A. melegueta* were purchased from Ilode Market in Ile-Ife, Nigeria. The plant was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where specimen copy was deposited and the voucher number 17524 was collected. The seeds were removed from the stalks, sun-dried and ground into fine powder using Manual Blender.

Experimental animals

Thirty albino rats with average weight (147 ± 4) g were purchased from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. They were housed in standard plastic cages under laboratory conditions with alternating light and dark cycle of 12 h each. They had free access to feeds (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. The rats were acclimatized for three weeks before the commencement of the experiment.

Reagents and chemicals

All reagents used were of analytical grade from Sigma-Aldrich Company and British Drug House (BDH) Chemical Limited England, Fine Chemical Limited Upsalla, Sweden, Fluka Chemical Company and Sigma Chemical Company, St Louis, M. U.S.A. Diagnostic Kits for the assay of total protein concentration was obtained from Randox Laboratory Ltd. Antrium, U.K. All buffers, reagents and solutions were prepared with glass distilled water and stored in the refrigerator.

Preparation of methanolic seed extracts of *Aframomum melegueta*

The defatted seed residue (250 g) was extracted by adopting earlier procedure of Oyedapo and Amos (1997). The powdered seed was soaked in 900 ml of 70% (v/v) methanol for 24 h. The suspension was squeezed and followed by filtration to obtain methanolic extract using Whatmann's No 1 filter paper. The residue was re-soaked and re-extracted 5 more times with 70% (v/v) methanol until the filtrate becomes colourless. The filtrates were combined, filtered and subjected to evaporation on a rotatory evaporator (Buchi Vacuum Pump, v-700 and Rotavapor RII, Switzerland) at 45°C to obtain syrup termed methanolic seed extract of *A. melegueta* (MEAM).

Phytochemical screening of the extract

Chemical tests for the identification of the secondary metabolites in the MEAM were carried out according to a procedure that was based on the methods of Oyedapo et al. (1999) and Sofowora (2002). (a) alkaloid with Drangendorf's reagent and Mayer's reagent, Wagner's reagent; (b) flavonoids with 10 ml of ethylacetate and 1 ml of dilute ammonia solution; (c) tannins with 10 ml of distilled water and a few drops of 0.5 M Ferric chloride in glacial acetic acid; (d) cardiac glycosides with 2 ml of chloroform and few drops of sulphuric acid (H_2SO_4) carefully layered at the bottom of the filtrate to form a lower layer interphase; (e) terpenoids with 10 ml of chloroform and 2 ml of concentrated sulphuric acid; (f) steroids/phytosterols with 2 ml of acetic anhydride and 2 ml of H_2SO_4 ; (g) saponins with 4 ml of distilled water and subjected to vigorous shaking for froth formation; (h) xanthoproteins and phlobatanins with 5 ml of distilled water, few drops of nitric acid, ammonia solution and 2 ml 10% (v/v) HCl.

Evaluation of antioxidant potentials of MEAM

The evaluation of the antioxidant potentials of methanolic extract of *Aframomum melegueta* involved:

(a) Assay of reducing power: The reducing power of MEAM was carried out according to a procedure based on the method of Oyaizu (1986). A higher absorbance indicated a higher reducing power.

(b) Estimation of total flavonoid contents (TFC): The total flavonoid content of the extract was estimated according to a modified method of Singh et al. (2010). The total flavonoid content was expressed in milligram rutin equivalent (RE)/g extract.

(c) Estimation of total phenolic contents (TPC): The total phenolic content of the extract was estimated according to the method of Singleton et al. (1999). The concentration of phenolic was extrapolated from the standard curve and expressed in milligram tannic acid equivalent (TAE)/g extract.

(d) Estimation of Vitamin C content: The concentration of Vitamin C in the MEAM was estimated spectrophotometrically according to a procedure based on those of Omaye et al. (1979) and Japota and Dani (1982). The vitamin C content of the extract was extrapolated from ascorbic acid standard calibration curve and was expressed in milligram per gram (mg/g) extract.

(e). Estimation of the Vitamin E content: The vitamin E content in the methanolic extract was estimated according to the method of Baker and Frank (1968) as slightly modified by Santhosh et al. (2013). The total vitamin E was expressed in milligram trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent per gram of extract (mg TE/g).

(f) Assay of DPPH radical scavenging activity: Assay of 1, 1-Diphenyl-2-picrylhydrazyl radical scavenging activity of MEAM was carried out according to a procedure based on the methods of Blois (1985) as reported by Cakir et al. (2011) and Bode and Oyedapo (2011). The percentage free radical scavenging activities of the standard and MEAM were calculated using the percentage inhibition of DPPH.

I_{DPPH} % was then plotted against the concentrations of MEAM and a logarithmic regression curve was established in order to calculate the IC_{50} value.

Evaluation of biological activity of extract of *Aframomum melegueta*

Evaluation of in vitro anti-inflammatory potential of extract of *Aframomum melegueta*

The red blood cell membrane stabilizing model exposed to both heat and hypotonic induced lyses was employed to evaluate the anti-inflammatory potential of the MEAM. The assay was carried out according to the procedures earlier reported (Oyedapo et al., 2015; Bode and Oyedapo, 2011).

The percentage membrane stability was calculated as:

$$\text{Percentage (\%)} \text{ Membrane Stability} = 100 - \frac{(Abs_{test} - Abs_{drug})}{Abs_{blood\ control}} \times 100$$

where Abs = Absorbance.

Grouping and treatment of experimental animals

Monosodium glutamate (2 g/kg bwt) was dissolved in 1% (v/v) DMSO and administered orally for 21 consecutive days according to the procedure earlier reported by Ramanathan et al. (2007). The animals were kept fasted 2 h before drug administration. The experimental rats were divided into six (6) groups of five (5) animals each and treated as follows:

Group I: Served as control and received 1% (v/v) DMSO.

Group II: Rats + Monosodium glutamate (2 g/kg bwt).

Group III: Rats + Monosodium glutamate (2 g/kg bwt) + Extract (125 g/kg bwt).

Group IV: Rats + Monosodium glutamate (2 g/kg bwt) + Extract (250 g/kg bwt).

Group V: Rats + Monosodium glutamate (2 g/kg bwt) + Gabapentin (20 g/kg bwt).

Group VI: Rats + Extract (175 g/kg bwt).

The rats were treated daily with extract/drug for 21 days, 1 h after monosodium glutamate treatment. The animals were weighed weekly and changes in body weight were calculated by subtracting the weight of the animals on the last day treatment from the weights before the commencement of oral MSG administration. This was expressed as percentage change.

The rats were euthanized by cervical dislocation on the 22nd day. The brain tissues were dissected, excised, perfused in n-saline (0.85% NaCl) to remove blood, blotted with filter paper, weighed and kept frozen in the deep freezer.

Preparation of brain homogenates

The brain tissues were weighed, mince into bits with clean and sterile scissors and divided into 3 portions. A portion was used for the preparation of 10% (w/v) brain homogenate with 0.25 M sucrose and 0.2 M EDTA.Na in 0.2 M Tris-HCl buffer, pH 7.4. The brain homogenate was first centrifuged using a Bench Centrifuge Model 90-2, Microfield Instrument, Essex, England at 3500 g for 10 min. Then, the supernatants were carefully collected and the precipitate discarded. The supernatants were further centrifuged at 12,000 rpm for 15 min. at 4°C using a Cold Centrifuge Model 8880, Centurium Scientific LTD, West Sussex, U.K. The supernatants were then collected into clean vials and used for the assays of non-enzymatic and enzymatic anti-oxidants, evaluation of nitric oxide, metabolic enzymes and protein concentrations. The precipitate was re-washed twice to give a Mitochondrial Fraction (MF) and used for the estimation of monoamine oxidase (MAO) activity (Pan et al., 2005). A portion was used for the estimation of brain lipid profiles while the other portion was used in the assay of brain biogenic amine activity.

Biochemical analyses

Assay of brain marker enzyme activities

(i) Na^+K^+ -ATPase activity: The activity of Na^+K^+ -ATPase in the brain homogenates were assayed according to a procedure based on the methods of Sovoboda and Mossinger (1981) and Chen et al. (1956) as described by Ames and Dubin (1960). The assay mixture contained substrate (250 μ l) and 50 μ l of brain homogenate was incubated at 37°C for 1 h. The reaction was terminated by the addition of 250 μ l 10% (w/v) trichloroacetic acid (TCA). The cooled suspension was centrifuged at 3500 rpm for 10 min. The inorganic phosphate concentration was estimated using the supernatant as reported (Ames and Dublin, 1960). The brain homogenate Na^+K^+ -ATPase activity was calculated in nmole of Pi liberated/min/mg protein.

(ii) Assay of Acetylcholinesterase (AChE) activity: The acetylcholinesterase (AChE) activity was assayed according to a procedure based on the method of Ellman et al. (1961). Typically, 2.6 ml phosphate buffer (0.1 M, pH 8.0) was mixed with 0.1 ml (0.01 M DTNB) followed by the addition of 40 μ l brain homogenate and incubated for 5 min. at room temperature. The reaction was initiated by the addition of 40 μ l (0.075 M acetylthiocholine iodide). The rate of hydrolysis of ATChI was monitored spectrophotometrically by

measuring the change in the absorbance per minute ($\Delta A/\text{min}$) at 420 nm over a period of 3 min. at 30 s interval. The enzyme activity was expressed in $\mu\text{mol}/\text{min}/\text{mg}$ protein and calculated as follows:

$$\text{Enzyme Activity } (\mu\text{mol SH hydrolysed}/\text{min}/\text{mg protein}) = \frac{\Delta A \times T_v}{L \times S_v \times \epsilon_{\text{DTNB}}}$$

where: ΔA = change in absorbance/minute; T_v = total volume of reaction mixture; S_v = volume of test sample in reaction mixture; ϵ_{DTNB} = extinction coefficient of DTNB $1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; and L = light path length (1 cm).

(iii) Assay of glutamine synthetase activity: The activity of glutamine synthetase was assayed according to a procedure based on the methods of Sadasivam and Manickam (2003). Typically, 500 μl substrate and 100 μl brain homogenate were incubated at 37°C for 5 min. The reaction was terminated by the addition of 500 μl (10% (w/v) of TCA. The cooled suspension was centrifuged at 3500 rpm for 10 min. The inorganic phosphate concentration in the supernatant was estimated as reported above.

The glutamine synthetase activity was calculated in nmole of Pi liberated/min/mg protein.

(iv) Assay of monoamine oxidase (MAO) activity: The monoamine oxidase activity was assayed according to the procedure based on the methods of Pan et al. (2005) with slight modification. To 100 μl of substrate (4 mM 5-hydroxytryptamine) was added 250 μl (mitochondrial fraction). The reaction mixture was incubated at 37°C for 20 min. and terminated by the addition of 200 μl (1 M HCl). The reaction product was extracted with 5 ml butyl acetate, mixed thoroughly and allowed to separate into two layers. The absorbance of the upper layer (organic phase) was read at 280 nm against the reagent blank.

The MAO activity was expressed in $\mu\text{mole}/\text{mg}$ protein and calculated as follows:

$$\text{MAO activity } (\mu\text{mol}/\text{mg protein}) = \frac{\text{Abs}_{280} \times T_v}{L \times S_v \times \epsilon}$$

where: Abs = Absorbance; T_v = total volume of reaction mixture; S_v = volume of test sample in reaction mixture; ϵ = $12.81 \text{ mmol}^{-1}\text{cm}^{-1}$; and L = light path length (1 cm).

Evaluation of brain enzymatic anti-oxidant activities

(i) Assay of glutathione peroxidase (GPx) activity: The assay of glutathione peroxidase (GPx) activity was assayed according to a procedure based on the methods of Rotruck et al. (1973). The Gpx activity was estimated and expressed as μmol of GSH consumed/min/g Hb.

(ii) Assay of catalase activity: Activity of catalase (EC.1.11.1.6) was assayed according to a procedure based on the methods of Sinha (1972). The method was based on catalase decomposing hydrogen peroxide to form water. The catalase activity was estimated and expressed in $\mu\text{mol}/\text{min}/\text{mg}$ protein.

(iii) Assay of superoxide dismutase (SOD) activity: Assay of superoxide dismutase activity was assayed according to the procedure based on the method of Misra and Fridovich (1972). The SOD activity was expressed as percentage inhibition of adrenaline oxidation in Unit/min/mg Protein.

Evaluation of non-enzymatic antioxidant in the brain

(a) Estimation of level of reduced glutathione (GSH): The level

of reduced glutathione (GSH) content was quantified according to the procedure based on the method of Moron et al. (1979). The level of brain GSH content was expressed in μg GSH/ml sample.

(b) Estimation of nitric oxide (NO) level: The nitric oxide (NO) levels in the brain homogenate were measured as total nitrite and nitrate levels with the use of Greiss reagent by the method of Moshage et al. (1995). The level of brain nitric oxide content was expressed in $\mu\text{mol}/\text{mg}$ of nitrite.

(c) Estimation of the Vitamins C content: the concentration of Vitamins C in the brain homogenate was estimated spectrophotometrically according to procedure based on methods of Omaye et al. (1979) and Japota and Dani (1982). The vitamin C content of the extract was expressed in milligram per gram (mg/g) of extract.

(d) Estimation of the Vitamins E content: The Vitamins E content of the brain homogenate was estimated according to the methods of Baker and Frank (1968) and Santhosh et al. (2013). The Vitamin E content was expressed in milligram trolox equivalent per gram of extract (mg TE/g).

(e) Assay of lipid peroxidation activity: The thiobarbituric acid reactive species (TBARS) production in the brain was carried out according to a procedure based on the method of Su et al. (2009) with slight modification. MDA levels was calculated and expressed as $\mu\text{mol}/\text{g}$ tissue.

Histological studies of brain samples

The rats were sacrificed and the whole brain removed. One rat per each group was picked at random for histological analyses. The histopathological analyses of the brain tissues were carried out at Gbela Laboratories, Oluwalose Quarters, Road 7, Ile-Ife. The slides were examined and interpreted by Dr. O. A. Ayannuga at the Department of Anatomy and Physiology, Obafemi Awolowo University, Ile-Ife, Osun State.

Statistical analysis

Data were expressed as mean \pm SEM for the five rats in each group. Differences between the control and treated groups were determined by the One-way Analysis of Variance (ANOVA), using GraphPad 3 (prism). Significant difference level was considered if $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemicals are biologically active compounds that contribute immensely to protection against predators but they are also of great importance against diseases in man. Phytochemical screening of extract of *A. melegueta* revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids as demonstrated by the positive reactions exhibited by these molecules. The observation which was in agreement with the earlier reports of Echo et al. (2012). Flavonoids are mostly abundant polyphenols in the vegetables, fruits and seeds (Narayana et al., 2001). Saponins on the other hand have been shown to boost the immune system and lower the cholesterol concentration by competing against

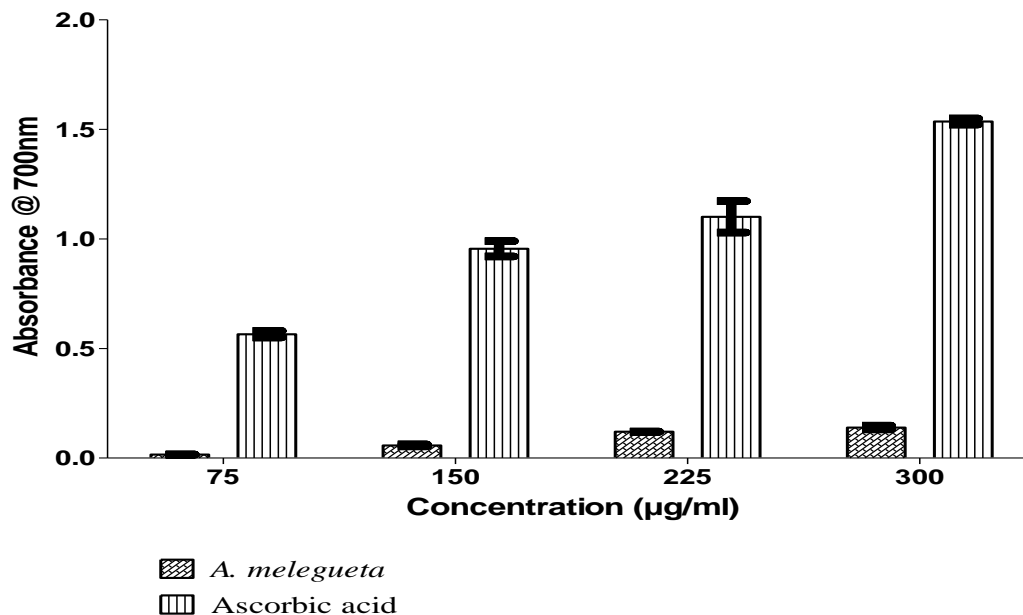


Figure 1. Reducing antioxidant power profile of *Aframomum melegueta* and ascorbic acid. Each value represented the mean \pm SEM of $n = 3$ reading. Value $p < 0.05$ was considered significant.

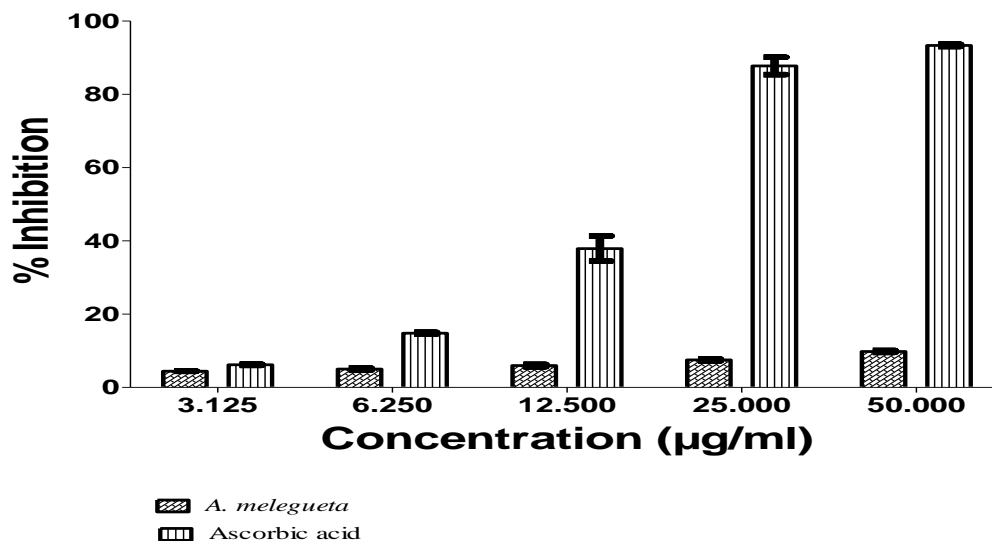


Figure 2. DPPH-radical scavenging activity of *Aframomum melegueta* and ascorbic acid. Each value represented the mean \pm SEM of $n = 3$ reading; $p < 0.05$ value was considered significant.

it for absorption in the body (Akinpelu et al., 2013). Tannins decrease protein quality by decreasing its digestibility and palatability, which causes damage to the intestinal tract and also interfere with iron absorption, which may result in carcinogenesis. Terpenes has also been implicated in the decrease of risk of cancer (Paduch et al., 2007). The reducing capacity of the plant extract serves as an indicator of its potential antioxidant activity (Gülçin et al., 2003).

In Figure 1, it was revealed that methanolic extract of

A. melegueta seed exhibited potent and appreciable reducing power, though lesser when compared with the reference standard compound (ascorbic acid), in a concentration dependent manner. Results revealed that methanolic seed extract of *A. melegueta* seed has appreciable DPPH radical scavenging activity with IC_{50} value of 306.84 $\mu\text{g/ml}$ while ascorbic acid (reference standard compound) gave IC_{50} value of 15.25 $\mu\text{g/ml}$ (Figure 2). The membrane stabilizing activity of MEAM on erythrocytes exposed to both heat and hypotonic induced

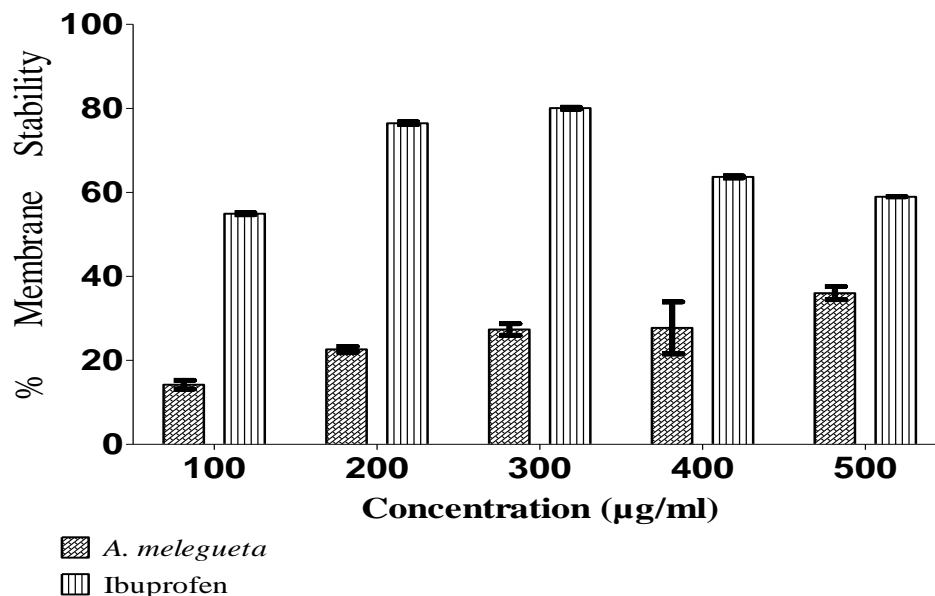


Figure 3. Membrane stabilizing activity of methanolic extract of *A. melegueta* and ibuprofen. Each value represented the mean \pm SEM of $n = 3$ reading; $p < 0.05$ value was considered significant.

Table 1. Effect of MSG, *Aframomum melegueta* extract and gabapentin on brain homogenate protein concentrations.

Group	Brain homogenate (g/dl)
I: Normal control	2.88 \pm 0.09
II: MSG (2 g/kg bwt) only	3.40 \pm 0.05 ^a
III: MSG + 125 mg/kg bwt extract	3.11 \pm 0.05 ^b
IV: MSG + 250 mg/kg bwt extract	3.04 \pm 0.03 ^b
V: MSG + 20 mg/kg bwt gabapentin	3.17 \pm 0.05 ^b

Each value represented Mean \pm SEM, $n = 5$ reading; $p < 0.05$ value was considered significant. The values with superscript (a) was significantly different from normal control, (b) was significantly different from MSG treated group, while (c) was significantly different from gabapentin treated group.

lyses showed a dose dependent activity over the concentration ranges tested. The result (Figure 3) revealed that MEAM exerted a response which was monophasic with maximum percentage stability of $36.02 \pm 1.56\%$ at $500 \mu\text{g/ml}$. The reference standard drug (ibuprofen), a non-steroidal anti-inflammatory drug, exhibited a biphasic response with maximum percentage stability of $80.04 \pm 0.34\%$ at $300 \mu\text{g/ml}$. The results obtained above are indicators of the extract potential ability in ameliorating altered level of certain biochemical parameters.

In Table 1, administration of MSG caused an increase (18.06%) in the brain protein concentration when compared to the control group. The result might be attributed to increase in brain concentration of glutamate as a result of MSG intake, resulting in increased level of

protein synthesis from the amino acid. Treatment with gabapentin, 125 and 250 mg/kg bwt extract significantly reduced protein concentrations by 7.26, 9.32 and 11.84% respectively when compared with MSG alone treated group.

In Table 2 is the summary of the effect of MSG, MEAM and Gabapentin on the activities of brain biomarker enzymes. In this present study, it was observed that MSG decreased the membrane bound enzyme $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity by 61.31%, which was significant ($p < 0.05$) when compared to the normal control group. This result was in agreement with previous studies that stated that based on anatomical co-localization, glutamate transporters are directly coupled to $\text{Na}^+\text{-K}^+\text{-ATPase}$ with increase in one depleting the level of the other (Erin et al., 2009). The depleted activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ thereby reduced the

Table 2. Effect of MSG, *Aframomum melegueta* extract and gabapentin on the activity of brain marker enzymes.

Group	Na ⁺ -K ⁺ -ATPase ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	Acetylcholinesterase ($\mu\text{mol}/\text{min}/\text{mg}$ protein) ($\times 10^{-2}$)	Glutamine Synthetase ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	Monoamine Oxidase ($\mu\text{mol}/\text{mg}$ protein)
I: Normal control	44.70 \pm 1.54	8.49 \pm 0.37	2.37 \pm 0.35	55.58 \pm 1.93
II: MSG (2 g/kg bwt) only	27.71 \pm 0.54 ^a	28.90 \pm 4.13 ^a	7.56 \pm 0.17 ^a	73.84 \pm 1.19 ^a
III: MSG + 125 mg/kg bwt extract	35.85 \pm 0.94 ^{bc}	13.54 \pm 0.54 ^b	5.05 \pm 0.15 ^{bc}	67.24 \pm 0.56 ^{bc}
IV: MSG + 250 mg/kg bwt extract	40.74 \pm 0.55 ^b	12.58 \pm 0.49 ^b	3.65 \pm 0.07 ^b	60.55 \pm 0.96 ^b
V: MSG + 20 mg/kg bwt gabapentin	42.00 \pm 1.02 ^b	11.93 \pm 0.29 ^b	3.42 \pm 0.15 ^b	57.42 \pm 0.81 ^b

Each value represented Mean \pm SEM, n = 5 reading; p < 0.05 value was considered significant. The values with superscript (a) was significantly different from normal control, (b) was significantly different from MSG treated group, while (c) was significantly different from gabapentin treated group.

energy that could be derived from hydrolysis of ATP to pump Na⁺ out of and K⁺ into the cell resulting in accumulation of glutamate in the extracellular compartment of the astrocytes, and alterations in Na⁺-coupled transport systems, osmoregulation and ion concentrations in excitable tissues. The treatment with 20 mg/kg bwt gabapentin, 125 and 250 mg/kg bwt extract resulted in increase of Na⁺-K⁺-ATPase activity by 51.57, 29.38 and 47.02% respectively. The methanolic seed extract of *A. melegueta* and gabapentin were able to activate and restore the Na⁺-K⁺-ATPase activities of the brain. The ability of the extract and reference standard drug to activate the enzyme activity might be due to decreased glutamate levels or blockade of glutamate action on the post-synaptic neurons and/or upregulation of Na⁺-K⁺-ATPase expression.

Acetylcholinesterase (AChE), an important cholinergic marker which terminates synaptic transmission at cholinergic synapses by rapidly hydrolyzing acetylcholine, was significantly (p < 0.05) increased by 240.40% in MSG - treated group when compared with the normal control group. The increase could be attributed to MSG administration causing a decrease in the

sensitivity of AChE to acetylcholine (lower pD₂) (Lobato et al., 2010). It was noted that treatment with 20 mg/kg bwt gabapentin slightly reduced the activity of acetylcholinesterase (58.72%) when compared with the MSG - treated group.

Moreover, administration of 125 and 250 mg/kg bwt extract also slightly reduced acetylcholinesterase activity (53.15 and 56.47%) respectively. This was in agreement with the findings of Adefegha and Oboh (2012a) that the inhibitory effect of *A. melegueta* seed extract on acetylcholinesterase activity could be attributed to the combined effect of the extract's phenolic and non-phenolic constituents. The result was also in agreement with the findings of Lucinei et al (2000), and Sowmya and Sarada (2015) that the increased in AChE activity in the hippocampus of MSG treated group only was as a result of dysfunction in the cholinergic system, which resulted in alteration of the enzyme.

There was 218.99% increase in glutamine synthetase activity in MSG treated group, which could be attributed to increase in glutamate level resulting from administration of MSG and the resulting glutamate was metabolized to glutamine by glutamine synthetase in the astrocytes. Moreover, treatment with 20 mg/kg bwt

gabapentin moderately reduced glutamine synthetase activity by 54.57%, which was in agreement with the observations of Leach et al. (1997) that chronic gabapentin administration caused a decrease in glutamate level and thereby, decrease in glutamate synthetase activity. The treatment with 125 and 250 mg/kg bwt extract significantly inhibited the enzyme activity by 33.20 and 51.72% respectively. This might be seen as the extract mimicking the mechanism of action of gabapentin.

Monoamine oxidase is a mitochondrial membrane-bound enzyme which catalyzes the oxidative deamination of monoamines (Linardaki et al., 2013), thus regulating monoaminergic neurotransmission. The administration of MSG (2 g/kg bwt) caused a significant (p < 0.05) increase of 32.85% in MAO activity, when compared with the normal control group. The increase in MAO activity could be attributed to reduction in monoamine level after treatment with MSG (Heal et al., 1992). The treatment with gabapentin (20 mg/kg bwt), 125 and 250 mg/kg bwt extract reduced the activated MAO significantly (p < 0.05) by 28.60, 9.82 and 21.95% respectively, when compared to the MSG - treated group. This amelioration exhibited by the gabapentin and

Table 3. Effect of MSG, *Aframomum melegueta* extract and gabapentin on the antioxidant status of the rat brain tissues.

Group	GPx (unit/mg protein)	Catalase ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	SOD (unit/min/mg protein)	GSH (mg/g)	Nitric Oxide (μmol)	Vitamin C (mg/g)	Vitamin E ($\mu\text{g}/\text{mg}$)	Malondialdehyde (μmol)
I	2.46 \pm 0.09	4.42 \pm 0.09	0.68 \pm 0.03	48.34 \pm 2.92	15.88 \pm 0.07	1.00 \pm 0.01	82.20 \pm 0.06	2.80 \pm 0.08
II	1.71 \pm 0.03 ^a	1.28 \pm 0.08 ^a	0.26 \pm 0.03 ^a	14.60 \pm 0.45 ^a	18.15 \pm 0.03 ^a	0.91 \pm 0.03	78.40 \pm 0.05	4.77 \pm 0.11 ^a
III	1.98 \pm 0.04 ^b	2.43 \pm 0.12 ^{bc}	0.43 \pm 0.02 ^{bc}	18.22 \pm 1.00	16.37 \pm 0.03 ^b	1.17 \pm 0.02 ^{bc}	88.00 \pm 0.06 ^b	3.58 \pm 0.05 ^{bc}
IV	2.20 \pm 0.012 ^b	3.43 \pm 0.09 ^{bc}	0.57 \pm 0.04 ^b	23.32 \pm 0.21 ^{bc}	16.12 \pm 0.03 ^b	1.46 \pm 0.06 ^{bc}	94.80 \pm 0.28 ^b	2.91 \pm 0.07 ^b
V	2.16 \pm 0.03 ^b	1.67 \pm 0.12	0.58 \pm 0.02 ^b	13.41 \pm 2.25	16.29 \pm 0.01 ^b	1.70 \pm 0.04 ^b	92.00 \pm 0.08 ^b	2.96 \pm 0.08 ^b

Each value represented Mean \pm SEM, n = 5 reading; p < 0.05 value was considered significant. The values with superscript (a) was significantly different from normal control, (b) was significantly different from MSG treated group, while (c) was significantly different from gabapentin treated group.

extract might indicate restoration of monoamine levels depleted by MSG treatment.

On the effects of the treatment on the activities of enzymatic antioxidants (Table 3), it was observed that there was significant (p < 0.05) reduction (43.86%) in glutathione peroxidase activity when compared with the control Group I rats. The inhibition was in agreement with result of Rajagopal et al. (2013) who stated that treatment of cells with glutamate caused depletion of cellular glutathione peroxidase that preceded cell death. The treatment with gabapentin and the extract caused increases of 26.32, 15.79 and 28.65% in glutathione peroxidase activity, when compared with MSG - treated group, indicating the restoration of the enzyme activity.

Also catalase (CAT), an endogenous radical detoxifying antioxidant enzyme, was significantly (p < 0.05) reduced in MSG - treated group (245.31%) when compared with the control group. The decrease in the activity of catalase might be due to its utilization to scavenge reactive oxygen species (ROS) or glycation (Singh and Ahluwalia, 2003). The treatment with the extract significantly (p < 0.05) increased the enzyme activity (89.84 and 167.97%) while treatment with the gabapentin resulted in slight increase activity (30.47%).

Moreover, the activity of superoxide dismutase (SOD), an endogenous radical scavenging antioxidant enzyme, was significantly (p < 0.05) reduced in MSG - treated group (161.54%), when compared with the control group. The significant (p < 0.05) inhibition of SOD activity in the brain of rats treated with MSG might be as a result of increased flux of O₂⁻ radicals causing tissue damage or injury (Singh and Ahluwalia, 2003). The treatment with gabapentin and the extract significantly (p < 0.05) increased SOD activity by 123.08, 65.38 and 119.23% respectively, compared to MSG - treated group. This is an indication that the extract might have potentials in ameliorating alterations in enzymatic antioxidants.

The effect of the treatment on the levels of non-enzymatic antioxidants revealed that in MSG - treated group there was a significant (p < 0.05) reduction (231.10%) in GSH content when compared with the control group (Table 3). The depletion is in agreement with previous studies that stated that synthesis of glutathione in astrocyte involves glutamate, cysteine and glycine though excess of glutamate inhibits the transport of cysteine thereby blocking glutathione formation (Aissouni et al., 2002). Also it might be as a result of utilization of reduced glutathione by glutathione

peroxidase in detoxification of H₂O₂ generated by MSG administration (Srinivasan et al., 2015). The treatment with the extract caused increase (24.79 and 59.73%) in GSH content with only 250 mg/kg bwt extract showing significant (p < 0.05) difference when compared with the MSG - treated group. The extract might be acting as an antioxidant, thereby restoring the intracellular GSH level depleted due to oxidative stress posed by MSG administration. The increase in glutathione level with the extract treatment might be as a result of its involvement in the glutamergic system. On the other hand, treatment with gabapentin elicited a further decrease (8.87%) compared with MSG alone treated groups.

Nitric oxide (NO) is a highly reactive signal molecule in the CNS. In MSG - treated group there was an increase (14.29%) in brain NO content when compared with the normal control group. This is agreement with Lidija et al. (2007) who reported that, the increase of NO production in distinct brain regions functionally connected via afferents and efferent suggests that these regions are affected by neurotoxication. The treatment with gabapentin and extract caused decrease in the NO content (11.42, 10.87 and 12.59%) when compared with the MSG - treated group.

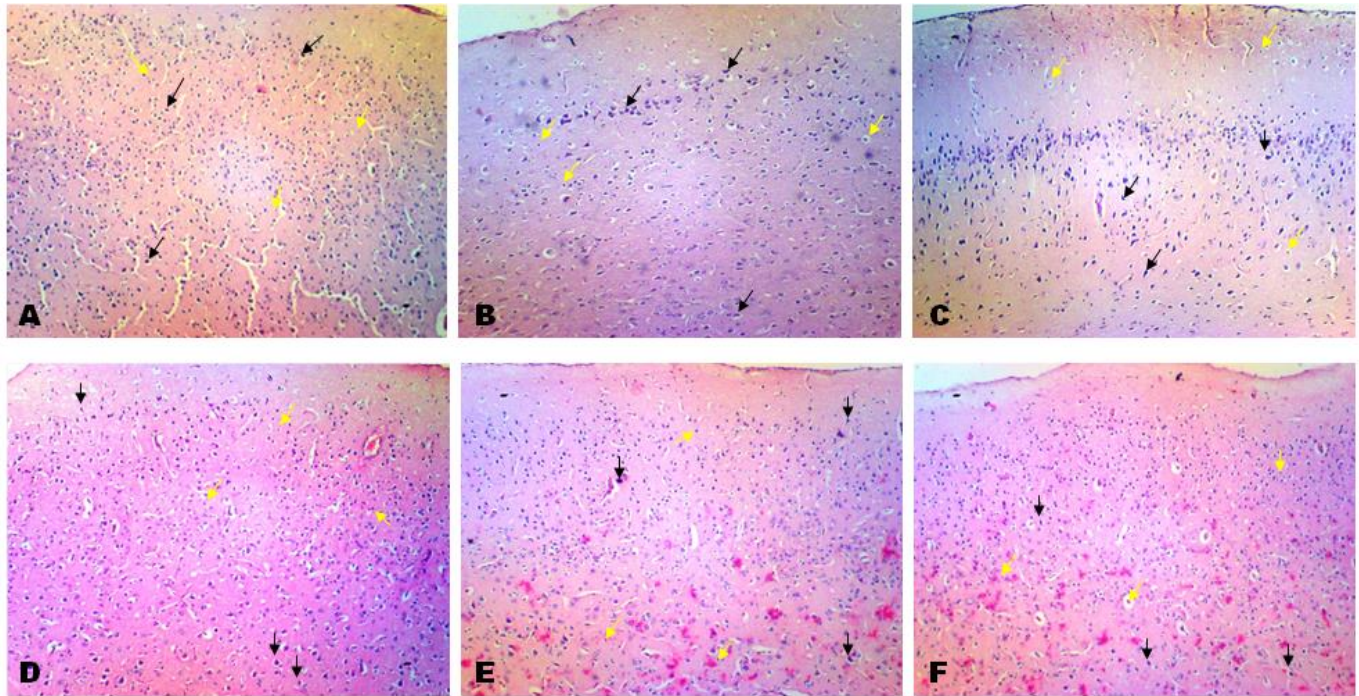


Plate 1. Photomicrographs of the cerebral cortical layers I to V of the different groups (A-F) showing the arrangement of cells (neurons-black arrows; glial cells-yellow arrows) in the different layers with clearly demarcated boundaries in A, B and C. Boundaries of layers are blurred in D, E and F. Mid-cortical regions of E and F showed patchy deep eosinophilic staining suggestive of cortical congestion. Max. X100, Stain; H&E. **A:** Section of the normal control group (I); **B:** Section of the brain of the MSG only group (II); **C:** Section of the brain of MSG + 125 mg/kg bwt extract; **D:** Section of the brain of MSG + 250 mg/kg bwt extract; **E:** Section of the brain of MSG + 20 mg/kg bwt gabapentin; **F:** 175 mg/kg bwt extract.

Moreover, the decrease in the nitric oxide level by the extract's downregulation of its activity might be attributed to the extract possession of strong antioxidant activities.

In the brain of MSG - treated there was a slight reduction (9.89%) in vitamin C content when compared to control group. The result might be due to neurons being able to maintain relatively high intracellular concentrations of vitamin C as internal antioxidant (May et al., 2006).

The treatment with gabapentin and extract significantly ($p < 0.05$) increased vitamin C contents by 86.81, 28.57 and 60.44% respectively when compared with MSG - treated group. Vitamin E content in MSG - treated group slightly decreased by 4.85%. This was in agreement with previous study of Traber and Stevens (2011) that Vitamin E was used up during its detoxifying action on MSG induced toxicity. However, treatment with gabapentin, 125 and 250 mg/kg bwt extract caused a significant increase (17.35, 12.24 and 20.92%) respectively in the Vitamin E content when compared with the MSG - treated group.

The MDA content of MSG - treated group when compared to normal control group elicited an increase (70.36%). The increase in lipid peroxidation observed might be attributed to a direct effect of increased generation of ROS resulting from MSG treatment. It was noted that treatment with gabapentin and extract caused

significant decrease of 61.15, 33.24 and 63.92% respectively in MDA content when compared with MSG - treated group.

Histological observations of brain sections showed an increase in ratio of astrocytes to neurons in MSG - treated group when compared with the control group (Plate 1). The increased ratio observed might be as a result of elevated astrocytes concentrations due to its requirement in ameliorating the toxic potency of glutamate. The increase was in agreement with Sriram et al. (2004) who reported that mechanical, chemical or degenerative insults to the brain stimulate astrocyte's proliferation and hypertrophy with increased synthesis of glial fibrillary acidic protein leading to vigorous astrogliosis. Also, Plate 1 showed that mid-cortical regions of the brains of experimental animals treated with MSG along with gabapentin elicited patchy deep eosinophilic staining indicating cortical congestion, though the neurons were unaffected. This was an indication that prolongs treatment with gabapentin exhibit toxic effects on brain tissues. The result agreed with previous study that chronic administration of gabapentin and carbamazepine caused increase in neurodegenerative changes in the adult brains (Olaiyi et al., 2014).

Plate 2 showed that cells in MSG - treated group are

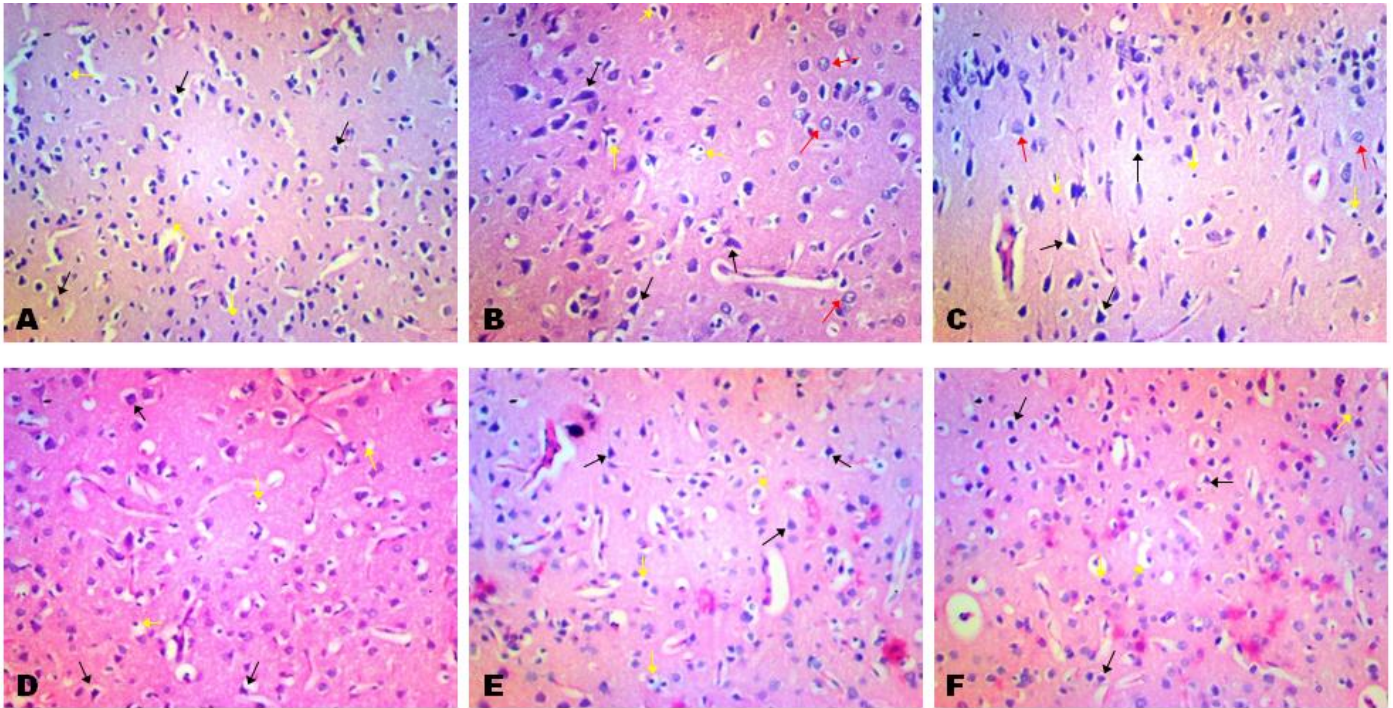


Plate 2. Photomicrographs of the cerebral cortical layers II and IV of the different groups (A-F) showing the arrangement of cells (neurons-black arrows; glial cells-yellow arrows) in the different layers. Patchy deep eosinophilic portions are noted around layer IV of group E and F suggesting cortical congestion. Cells in group B and C appear bigger than those of the other groups. Degenerating neuron (Red arrows) are noted in group B and to a lesser extent in C with vacuolated cytoplasm. Max. X 400, Stain: H&E. **A:** Section of the normal control group (I). **B:** Section of the brain of the MSG only group (II); **C:** Section of the brain of MSG + 125 mg/kg bwt extract; **D:** Section of the brain of MSG + 250 mg/kg bwt extract; **E:** Section of the brain of MSG + 20 mg/kg bwt gabapentin; **F:** 175 mg/kg bwt extract.

bigger than those of the other groups. This was an indication that glutamate administration resulted in degeneration of brain cells. The treatment with extract was able to ameliorate this effect, which might be due to the extract containing gingerols and related compounds that may be useful against degeneration (Obike et al., 2014). Loss of cerebral structure and degenerating neurons (Red arrows) with vacuolated cytoplasm were observed in MSG - treated group (Plates 1 and 2), indicating neuro-damaging effect of MSG. The observation was in agreement with previous study that stated that administration of MSG showed some decreased cellular population, degenerative changes, cellular hypertrophy and vacuolations which appeared in the brains of MSG-treated groups compared with the control group (Eweka and Om'Iniabohs, 2006). The treatment with extract and gabapentin protected the cerebral structure (Plates 1 and 2).

Conclusion

The study revealed that oral administration of MSG at dose level of 2 g/kg bwt elicited deleterious neurotoxicity. The altered levels of biochemical parameters and brain

biomarkers were adequately improved by treatment with extract. The reference standard drug was able to improve brain biomarkers but inefficient in the maintenance of the antioxidant biochemical parameters. The neuroprotective effect of the extract was also observed to be concentration dependent. Finally, the above neuroprotective, antioxidant and anti-lipid peroxidation effects of the extract of *A. melegueta* seeds might be the rationale behind some of its folkloric uses and also could be responsible for some of its pharmacological effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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