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"*Telfairia occidentalis* leaf extract mitigated Monosodium glutamate-induced behavioural and histological alterations in rat hippocampus"

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Abstract

Background: Monosodium glutamate (MSG) toxicity in rodents reportedly causes damage to the brain via oxidative stress. *Telfairia occidentalis* ethanolic extract (TOE) may mitigate this damage by virtue of its known antioxidant property. This study investigated the possible protective role of TOE against MSG-induced alterations in rat brain microanatomy.

Materials and methods: Forty two adult male rats (130-150 g) were randomized into six groups as follows: Group 1: Control, food and water; Group 2: TOE (400 mg/kg body wt.); Group 3: VIT E (500 mg/kg body wt.); Group 4: MSG (4 g/kg body wt.); Group 5: MSG (4 g/kg) + VIT E (500 mg/kg) and Group 6: MSG (4 g/kg) + TOE (400 mg/kg). MSG was given to induce neurotoxicity in rats one hour before treatment with TOE or VIT E. All treatments were given by gastric gavage for 14 days. Behavioural tests were conducted on day 15 and the rats were subsequently euthanized with i.p. Ketamine hydrochloride. The harvested brain tissues were fixed in neutral buffered formalin and hippocampal biopsies processed for light microscopy using haematoxylin and eosin stain. Granule and pyramidal neurons of hippocampus were assessed quantitatively.

Results: Monosodium glutamate significantly reduced the frequency of some behavioural tests while increasing others relative to control group. MSG induced degeneration of some granule and pyramidal neurons of the hippocampus and also significantly ($p<0.05$) reduced the neuronal count of both neuron types. Both MSG + VIT E and MSG + TOE co-treatments reversed the histologic alteration and significantly elevated neuronal density when compared with MSG group.

Conclusion: The ethanolic extract of *Telfairia occidentalis* demonstrated protective effects against MSG-induced histological alterations in rat hippocampus.

Keywords: *Monosodium glutamate, Telfairia occidentalis, dentate granule cells, CA3 pyramidal neurons, neurodegeneration.*

Résumé

Contexte : La toxicité du glutamate de mono-sodium (GMS) chez les rongeurs cause de manière constatée des dommages au cerveau par le biais du stress oxydatif. L'extrait éthanoïque de *Telfairia occidentalis* (ETO) peut atténuer ces dommages en raison de sa propriété antioxydant connue. Cette étude a examiné le rôle protecteur éventuel d'ETO contre les altérations induites par le GMS dans la micro-anatomie du cerveau du rat.

Matériels et méthodes : Quarante-deux rats mâles adultes (130-150 g) ont été randomisés en six groupes comme suit: Groupe 1: contrôle, nourriture et eau; Groupe 2 : ETO (400 mg / kg de poids corporel); Groupe 3: VIT E (500 mg / kg poids corporel); Groupe 4: GMS (4 g / kg de poids corporel); Groupe 5: GMS (4 g / kg) + VIT E (500 mg / kg) et groupe 6: GMS (4 g / kg) + TOE (400 mg / kg). On a administré le GMS pour induire une neuro-toxicité chez les rats une heure avant le traitement par ETO ou VIT E. Tous les traitements ont été administrés par gavage gastrique pendant 14 jours. Des tests comportementaux ont été effectués au jour 15 et les rats ont ensuite été euthanasiés avec le chlorhydrate de kétamine i.p. Les tissus cérébraux recueillis ont été fixés dans du formol à tampon neutre et des biopsies de l'hippocampe ont été traitées pour la microscopie optique en utilisant de la coloration à l' hématoxyline et à l'éosine. Les neurones granulaires et pyramidaux de l'hippocampe ont été évalués quantitativement.

Résultats: Le glutamate mono-sodique a réduit de manière significative la fréquence de certains tests comportementaux tout en en augmentant d'autres par rapport au groupe témoin. Le GMS a induit la dégénérescence de certains neurones granulaires et pyramidaux de l'hippocampe, ainsi qu'a significativement ($p <0,05$) réduit le nombre de neurones des deux types de neurones. Les deux co-traitements GMS + VIT E et GMS + ETO ont inversé

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l'altération histologique et ont significativement élevé la densité neuronale par rapport au groupe GMS.

Conclusion: L'extrait éthanoïque de *Telfairia occidentalis* a démontré des effets protecteurs contre les altérations histologiques induites par le GMS dans l'hippocampe de rat.

Motsclés: *Glutamate de mono sodium, Telfairia occidentalis, cellules granulaires dentées, neurones pyramidaux CA3, neuro-dégénérescence*

Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids [1]. Monosodium glutamate is present in fruits and vegetables such as tomatoes and potatoes but its safety as a food additive has generated controversies both locally and globally [2]. Glutamate is an excitatory neurotransmitter in the mammalian brain which plays important roles in both physiological and pathological processes [3]. It is released from the vesicles in presynaptic terminals by a calcium-dependent mechanism that involves voltage-dependent calcium channels and is also recognized as an excitotoxin [4-6]. Despite experimental reports of the toxicity of MSG, the Food and Drug Administration and Control (FDA) of the United States reported that MSG was safe [7], a position that might have encouraged its continued use by many restaurants and families. It has been reported to be neurotoxic [7] and the neurotoxicity has been attributed to the mechanism of oxidative stress among other factors [8, 9], however, the use of antioxidant complexes like vitamin C, vitamin E and Quercetin has reportedly ameliorated this [10, 11].

Telfairia occidentalis commonly called fluted pumpkin is a vegetable which belongs to the family Cucurbitaceae. In Nigeria, it is called "Ugu" (Igbos), "Aporoko" (Yoruba), "Ubong" (Efik), "Umee" (Urhobo), and "Umeke" among the Edo people [12]. Fluted pumpkin is majorly cultivated for its leaves and eaten as potherb, and the seeds eaten as "egusi soup". This vegetable is known to contain protein, carbohydrate, fat, calcium, iron, magnesium, potassium and vitamins such as A, B2, B5, B12 and thiamine thus possessing health-maintaining potentials [13, 14]. Phytochemically, it contains oxalates, saponins, glycosides, alkaloids, resins and flavonoids, the latter being responsible for its antioxidant and free radical scavenging properties [15-17]. Its use in the treatment of anaemia in pregnant women is premised on the high iron content of its leaves [18].

Alpha-tocopherol (vitamin E) is a natural occurring vitamin found in a variety of fruits and beneficial to overall body functions as well as possessing antioxidants property thus helping the body to combat degenerative conditions. It is an intracellular compound associated with lipid-rich biological membranes; it is lipophilic making it a major free radical chain terminator [19].

The mammalian hippocampus is located in the temporal lobe of the cerebrum and consists of the Ammon's horn or cornu ammonis regarded as the hippocampus proprius and the dentate gyrus [20]. It is important in memory formation and consolidation [21]. Memory may be in form of either declarative (semantic, episodic or spatial) or non-declarative [22]. Memory formation depends on the intact hippocampal formation consisting of entorhinal cortex, dentate gyrus, hippocampus proprius and the subiculum which is a transitional zone between the entorhinal and hippocampal cortices [21]. Before the hippocampus is involved in memory formation, sensory information must pass through a hierarchically organized neocortical network involving primary sensory cortices activation, supplemental sensory areas and high-order association cortices. Following these events, information is then fed to the entorhinal cortex which is associated with the trisynaptic circuit, a pathway that relays information from the perforant path to the dentate gyrus, dentate to cornu ammonis3 (CA3), and CA3 to cornu ammonis1 (CA1) [23-25]. Damage to any part of this pathway would affect the functions of the hippocampus due to structural alterations which monosodium glutamate might elicit since it has been associated with neuronal injury and death [6].

The objective of this study was to investigate the effect of possible protective property of *Telfairia occidentalis* leaf extract and alpha-tocopherol on the hippocampal neurons of male Wister rats that have been exposed to monosodium glutamate.

Materials and methods

Plant materials and extraction process

Telfairia occidentalis leaves were procured from a commodity market in Iwo, Nigeria. Botanical identification and authentication of the leaves were done at the herbarium of Department of Biological Sciences, Bowen University, Iwo, Nigeria where a voucher number: BUH08 was given and a specimen was deposited. The leaves were dried at room air, then processed into the powdery form. About 102.0g of the powder was used to obtain 11.7 g of the *Telfairia occidentalis* ethanolic extract (TOE) thus giving an 11.5 % yield.

Experimental Animals

Forty-two (42) adult male Wistar rats weighing between 130 g-150 g were acquired from the breeding colony and kept in the Animal House of the College of Health Sciences Medicine, Bowen University, Iwo, Nigeria. They were housed in netted wooden cages having dimensions 43 cm × 40 cm × 29 cm and soft wood shavings employed as bedding at room temperature in a 12 hour light/dark cycle. They were allowed to acclimatize for 4 weeks before randomization into different groups. They were fed with rat pellet diet and water *ad libitum*. Animal experiments were performed in accordance with the principle of humane care and use of laboratory animals [26].

Preparation and administration of monosodium glutamate

The Ajinomoto brand of monosodium glutamate (MSG) was used for the study. The crystals were dissolved in water and administered orally via cannula at a daily dose of 0.8 g/ml.

Administration of vitamin E (VIT E)

Vitamin E 100 mg capsules manufactured by Futurebiotic LLC, Hauppauge, New York, USA was used for the study. The content was aspirated with syringe and needle and a concentration of 500 mg / ml was administered to each animal orally.

Research design and animal grouping

The rats were randomized into 6 groups as shown below:

Group 1 (N=6): control group given food and water only; Group 2 (N=6): TOE (400 mg/kg); Group 3 (N=6): VIT E (500 mg/kg); Group 4 (N=8): MSG (4 g/kg); Group 5 (N=8): MSG (4 g/kg) + VIT E (500 mg/kg); Group 6 (N=8): MSG (4 g/kg) + TOE (400 mg/kg). All administrations were through oropharyngeal cannula and lasted 14 days as daily single doses. Dosages were based on published reports: TOE of 400 mg/kg [12]; VIT E of 500 mg/kg [27] and MSG of 4 g/kg [28]. More animals were randomized into the three MSG groups so as to take care of possible attrition secondary due to the effect of MSG administration.

Behavioural tests

On experimental day 15, the animals were subjected to behavioural tests namely: Open field test (to assess behaviour of rats), forelimb grip test (to assess forelimb muscle strength) and geotaxis test (to assess vestibular and cerebellar function) according to the methods described by Adebiyi *et al.* [29].

Open field test

A wide box approximately 120 cm by 120 cm with an open roof was used. The box had lines drawn horizontally and vertically forming square grids. Each animal was placed in the centre square quadrant and then left free to move around. The parameters checked for included frequency of grooming, rearing, line crosses and stretched attend posture with each animal allowed a period of 5 minutes. Thereafter, box was cleaned with 70% alcohol and dried before introduction of the next animal so as to reduce olfactory bias.

Forelimb grip test: The ends of a 1 metre long metal wire was placed on two stools with weights used to maintain the ends. The space between the stools on the floor had a soft cushioning surface should the animal lose its grip and fall down. The animal was gently placed on the metal wire so as to grasp it with the forelimbs supporting its body weight. It suspended itself in that position until it either fell down or used its hind limbs to support its weight. Duration of suspension for three trials was recorded and the mean taken with each rat allowed spending 2 minutes per trial.

Geotaxis test: A wooden slab was inclined at about 45° to the vertical surface and the animal placed on the slab at its upper end. Its downward movement was monitored to record the duration it moves down the slope before turning upward to climb up the slope. This maximum duration of attempt for each animal was 2 minutes. The mean of three trials was recorded.

Tissue extraction, tissue processing, histology and morphometry

Upon completion of the behavioural tests, the animals were euthanized same day by cervical dislocation and the harvested brains were processed for light microscopy using Haematoxylin and Eosin stain [30]. Images were acquired from the histological slides by means of a Sony Cybershot camera mounted on an Olympus microscope. The densities of the viable pyramidal neurons of the cornu ammonis3 (CA3) and granule neurons of the dentate gyrus of the hippocampus were measured using a microscope with a graticule mounted on a microscope according to published methods [31]. Briefly, the micrometer was calibrated with a stage micrometer slide with a customized 2 mm ruler engraved on it (Leitz, Wetzlar, Germany) using the eyepiece lens 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 calculated to be 0.11 mm^2.

The densities of the viable neurons on the histological slides were determined by counting the number of viable neurons observed within a given square area in a section while excluding pyknotic eosinophilic neurons [32].

Statistical analysis

The numerical results were expressed as means ± standard error of mean. They were analyzed using one way analysis of variance (ANOVA) with Microsoft Office Excel 2011 and GraphPad Prism software (GraphPad software version 5.01, San Diego CA, 2010). Differences were considered statistically significant at $P < 0.05$.

Results

General observation

Feeding habits in MSG-treated rats reduced along the course of the study. Rats in the control and TOE treated groups were the most active during the period of research study. A rat each was infected in both MSG + VIT E and MSG + TOE groups and were isolated from the groups. The animals given TOE only were observed to have more faecal drops in their cages than in other groups

Relative brain weight changes

The relative brain weight changes at the end of experiment were not statistically different across the groups as shown in figure 1.

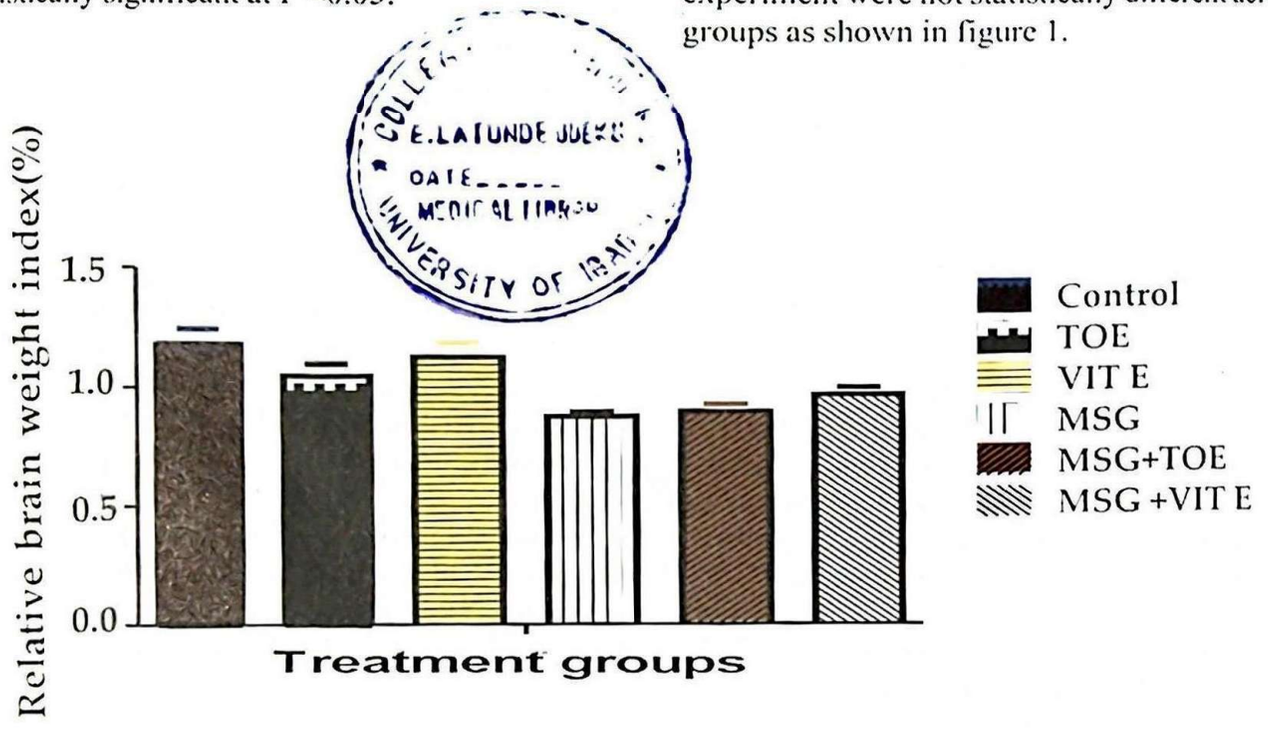

Fig. 1: Brain to body weight ratio of control and treated rats. Values were represented as mean ± S.E.M. of 5 rats. The alterations were not significantly different across the groups. MSG = Monosodium glutamate, TOE = *Telfairia occidentalis* ethanolic leaf extract, VIT E = Vitamin E.

Table 1: Effect of TOE and VIT E administration on the behavioural tests of male Wistar rats treated with MSG.

Group	LC	RE	GR	SAP	NGT (s)	FLG (s)
Control	48.80±13.9	12.20±2.31	18.20±4.42	7.20±1.23	2.33±0.32	33.74±5.52
TOE	35.00±2.70*	6.17±0.81*	39.83±19.81*	5.33±1.22	3.61±0.41	14.72±2.01*
VIT E	22.67±3.80*	6.83±2.42*	28.00±8.23*	6.83±1.52	5.39±0.51	52.45±19.03*
MSG	20.25±3.11*	5.50±1.31*	48.88±15.81*	7.63±1.13	5.13±0.52*	21.31±3.41*
MSG + TOE	18.67±6.02*	3.33±1.41	31.50±23.53#	5.67±2.13	4.56±0.53	16.33±3.21#
MSG + VIT E	17.50±2.91*	2.71±0.92#	47.57±22.41	5.00±0.91	5.56±1.13	43.61±12.42#

*Values are presented as mean ± S.E.M. of mean of 5 rats. TOE = Telfairia occidentalis ethanolic leaf extract, VIT E = Vitamin E, MSG = Monosodium Glutamate, LC = Line crossing, RE = Rearing, GR = Grooming, SAP = Stretch Attend Posture, NGT = Negative Geotaxis, FLG = Fore-Limb Grip. Posture. *P<0.05 versus Control. o&P<0.05 versus MSG*

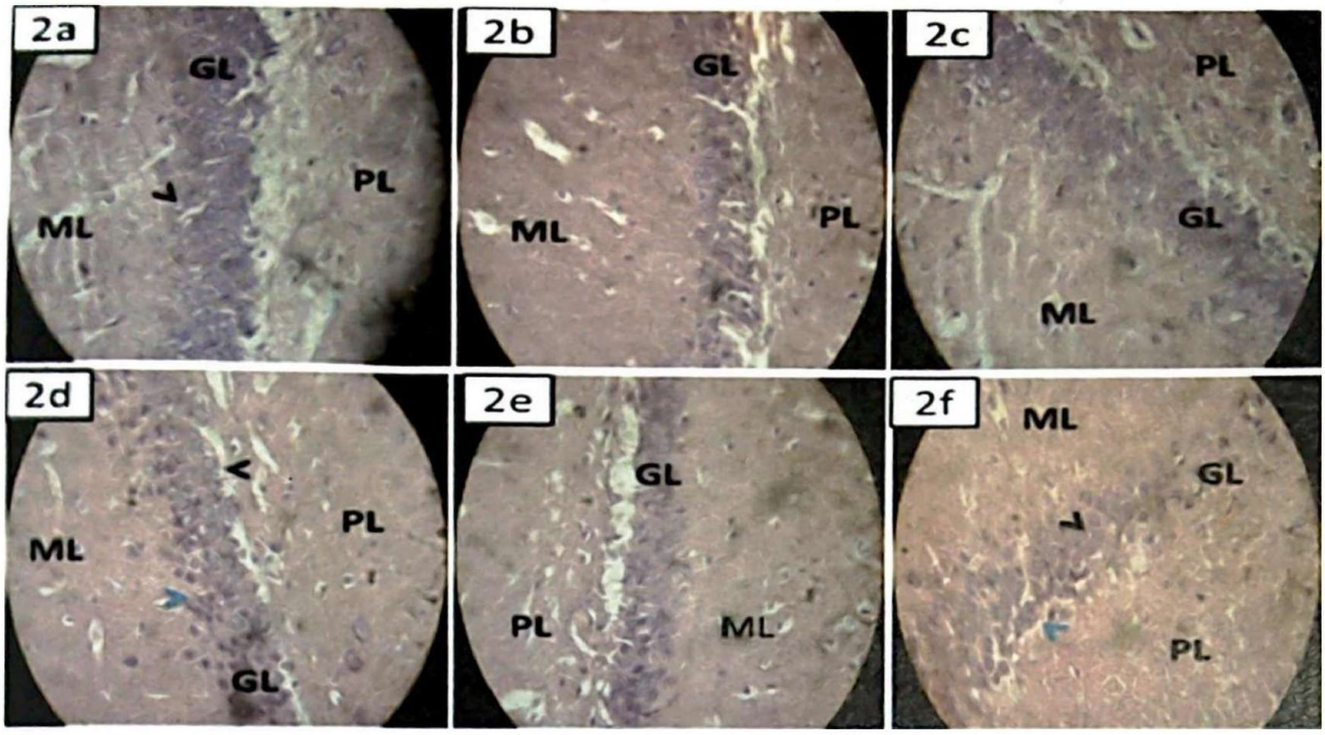

Fig. 2: Representative photomicrographs of dentate gyrus of rats. (A) Control (B) TOE (C) VIT E (D) MSG (E) MSG + TOE (F) MSG + VIT E. TOE = Telfairia occidentalis ethanolic leaf extract, VIT E = Vitamin E, MSG = Monosodium glutamate. MO = Molecular layer, GL = Granular layer, PL = Polymorphic layer. Black arrowhead = normal granule neurons, Blue arrowhead = degenerated granule neurons. H&E. X 768.

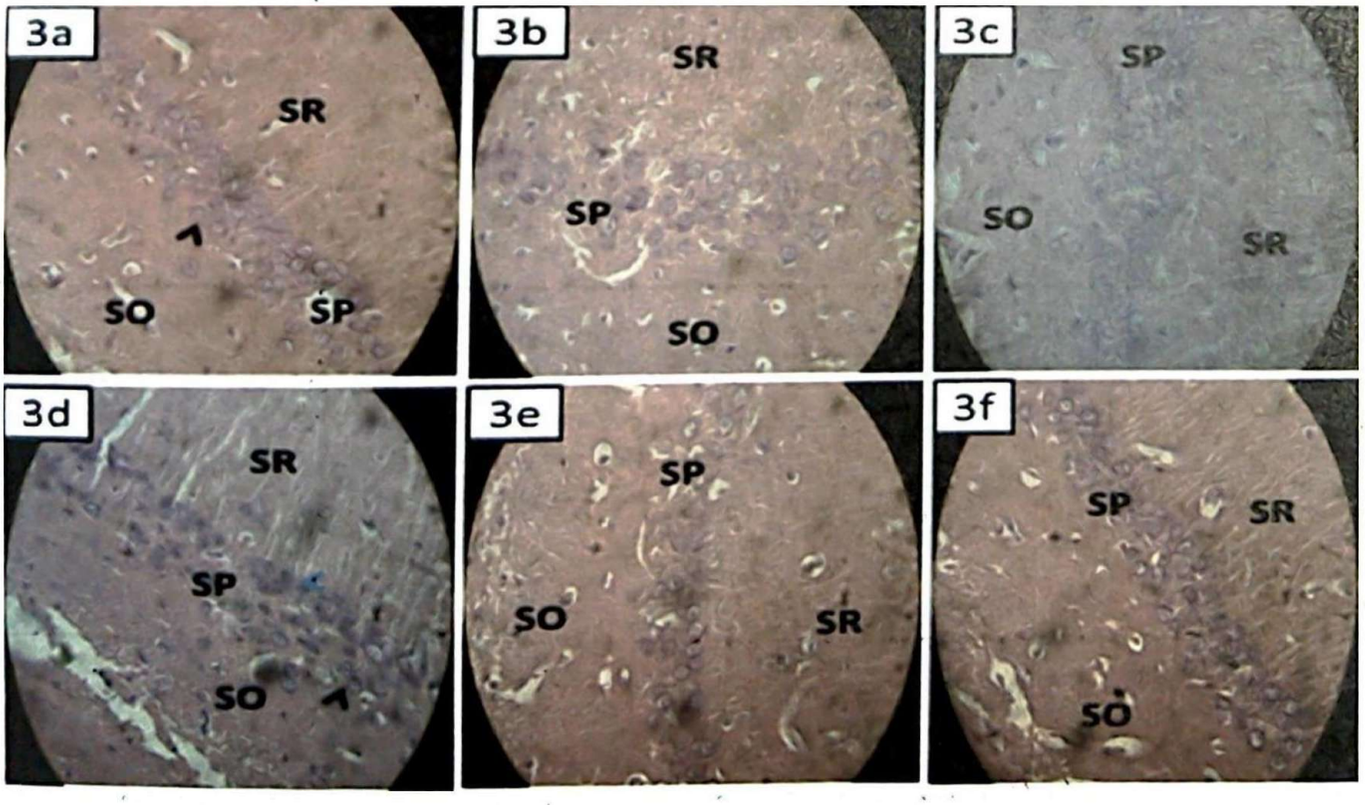

Fig. 3: Representative photomicrographs of cornu ammonis3 (CA3) field of hippocampus of rats. (A) Control (B) TOE (C) VIT E (D) MSG (E) MSG + TOE (F) MSG + VIT E. TOE = Telfairia occidentalis ethanolic leaf extract, VIT E = Vitamin E, MSG = Monosodium glutamate. SO = Stratum oriens, SP = Stratum pyramidalis, SR = Stratum radiatum. Black arrowhead = normal pyramidal neurons, Blue arrowhead = degenerated pyramidal neurons. H&E. X 768.

Behavioural tests

Table 1 showed that MSG significantly ($p<0.05$) reduced the frequency of LC, RE and the duration of FLG, but increased GR and NGT when compared with the control group. However, the concomitant treatment of MSG with TOE reduced all the parameters when compared with MSG treatment. In comparison, MSG+VIT E treatment increased FLG significantly ($p<0.05$) while reducing other parameters.

Histological evaluation of dentate gyrus tissue

Figure 2, shows that the layers of the dentate gyrus of the hippocampus namely: molecular, granular and polymorphic were well preserved in all groups. However, while the granule neurons in the control showed normal vesicular nuclei, some of the granule neurons in the MSG (Figure 2d) and MSG+VIT E (Figure 2f) groups showed some dark neurons scattered among the normal neurons.

Histological evaluation of cornu ammonis3 (CA3) tissue.

The photomicrographs of cornu ammonis3 of the hippocampus proprius (Figure 3) show a normal stratum oriens (SO), stratum pyramidalis (SP), stratum radiatum (SR) with normal microanatomic features in the control, TOE and VIT E groups. In the MSG group, various stages of degeneration (pyknosis and karyolysis) of pyramidal neurons of the SP layer were observed as shown in Figure 3d compared with the large vesicular nuclei of the control group.

Morphometric evaluation of hippocampal tissue

The density of viable pyramidal neurons of the CA3 and granule neurons of the dentate gyrus of the hippocampus were counted and displayed in Table 2. Both neurons were significantly ($p<0.05$) reduced by MSG relative to the control. In both MSG + VIT E and MSG + TOE co-treatment groups, density of viable neurons were significantly elevated when compared with MSG group as shown in table 2.

Discussion

This study investigated the effect of *Telfairia occidentalis* ethanolic extract (TOE) and alpha-tocopherol (VIT E) on monosodium glutamate (MSG) alterations of the behavioural and histology of rat brain. The organ-to-body weight ratio can be used as an index for assessing the state of an organ: a significant reduction in the value can be traced to organ or tissue necrosis, while a significantly high value is a possible indication of tissue inflammation [33]. In agreement with the reports of Abbas and Abd El-Haleem [34], there was no significant brain weight increase in the MSG-treated rats.

According to Hogas et al. [35], the open field test is used to evaluate the emotional state and locomotor activity of an animal and thus examines anxiety-related behaviour characterized by the normal aversion of the animal to an open area. Animals express anxiety and fear when removed from their acclimatized cage and placed in a new environment by showing alteration in behavioural parameters. Locomotor activity represents a broad class of sensory, motor and integrative processes [36] and central nervous system (CNS) depressants inhibit locomotor activity of animals, though other agents can excite the function of the CNS thus inhibiting sedation [37]. The significant reduction of line crossing and rearing (vertical movement) by MSG and TOE groups, when compared with control suggested a depressant-like activity by MSG which might be attributed to its toxicity. This observation regarding TOE was in agreement with report of Ajao and Akindele [12] indicating its sedative activity. The

Table 2: Effect of TOE and VIT E administration on mean densities of viable hippocampal neurons in male Wistar rats treated with MSG.

Groups	Density of CA3 pyramidal neurons(no/0.11mm^2)	Density of granule neurons (no/0.11mm^2)
Control	9.39±0.78	13.81±0.75
TOE	8.62±0.52	12.83±0.75
VIT E	8.93±0.83	12.82±1.12
MSG	4.91±1.41*	6.03±14.41*
MSG + TOE	9.07±0.56#	13.42±0.49#
MSG + VIT E	9.04±0.86#	13.23±0.75#

*Values are presented as mean ± S.E.M. of 5 rats. TOE = Telfairia occidentalis ethanolic leaf extract, VIT E = Vitamin E. MSG = Monosodium Glutamate. *P<0.05 versus Control. o&P<0.05 versus MSG.*

reduction in locomotor activities of rats by MSG + TOE and MSG + VIT E treatments groups suggested that these treatments were unable to ameliorate the effect of MSG on these parameters. The exhibition of a depressant-like activity by these agents on the CNS is in agreement with the report of Adebiyi *et al.* [29]. However, MSG + VIT E elevated the forelimb grip strength of the rats suggesting increased muscular strength. The increased frequency of grooming by MSG, MSG + TOE and MSG + VIT E treatment suggested absence of anxiety in the animals in agreement with the report of Umukoro *et al.* [38] who reported the absence of anxiety following MSG administration.

The duration a rat was able to hold on to the hanging wire is considered to be an indirect measure of grip, muscle strength and co-ordination which was significantly ($p<0.05$) increased in the VIT E group but reduced in the MSG group. This may suggest increased grip strength in the VIT E group, however, the reduction in the MSG group could be due to alteration in motor coordination and muscle tone [29]. Prolongation of time spent in negative geotaxis suggested the possibility of reduced vestibular sensitivity integrity and motor coordination in MSG-treated rats since ability to quickly turn uphill along the plane is dependent on an undisturbed vestibular system for body balance [39].

The histology of the hippocampus of the MSG group demonstrated neuronal alteration as evidenced by the presence of degenerating pyramidal neurons in the cornu ammonis3 (CA3) subfield of the hippocampus and those of granule cells of the granule layer of the dentate gyrus of hippocampal formation. This is in comparison to the histology of the CA3 and dentate gyrus (DG) of the control group which showed distinct large soma with vesicular nuclei and visible nucleoli in the pyramidal and granule neurons. In addition, the number of layers of the CA3 neurons was observed to have reduced (Figure 2). The hippocampus has been associated with roles in emotions, behaviour and memory (episodic, semantic and spatial) and damage to the hippocampus bilaterally would affect emotional behaviour (especially those related to pain) and inability to form new long-term memories [24, 22]. The consequence of degeneration or damage to granule cells by MSG as shown histologically would be an alteration of the quality of the excitatory projections of the entorhinal cortex (EC) layer II cells with the apical dendrites of DG granule cells from which Mossy fibres project to synapse with CA3 neurons [23].

Furthermore, as part of the trisynaptic pathway, impulses project from CA3 via its glutamatergic Schaffer collaterals onto ipsilateral CA1 pyramidal neurons thereby completing the hippocampal trisynaptic circuit [24, 25]. Damage by MSG to the pyramidal neurons as in this experiment could have affected the projections emanating from CA1 to the subiculum and entorhinal cortex layers IV and V neurons, which in turn project to superficial layers or high-order association cortices. To sum up, there might be reduction or alteration in the proper functions of these hippocampal parts which was partly demonstrated in this study by increased grooming and behavioural (reduced locomotion and rearing) alterations. Furthermore, acquisition and recall of episodic and spatial memories might be affected in these animals [22]. The histologic demonstration of protection of both granule and pyramidal neurons by TOE and VIT E when co-treated with MSG, however, suggested that normal hippocampal function in the rats of those groups might be restored thus supporting the hypothesis that TOE and VIT E can ameliorate the damaging effect of MSG on hippocampal neurons in this present study.

The toxicity attributed to MSG was (among other factors) generated via oxidative stress [8, 6], and it has been shown that substances with antioxidant capabilities can neutralize or reduce the oxidative damage of MSG [10, 11]. Although biochemical tests were not conducted in this present study, TOE and VIT E have been reported to possess antioxidant activities and able to scavenge free radicals, quench singlet and triplet oxygen and decomposing peroxides [15, 16, 27]. We therefore, reasonably assume that in part, the antioxidant activities of both TOE and VIT E might have participated in mitigating the MSG-induced damage observed in the hippocampus of rats thus mitigating the neural effects we observed and hence reduce or prevent all the potential associated consequences of the lesions on the microanatomy and function of the hippocampus of the brain.

Conclusion

Taken together, MSG elicited behavioural changes and histological alterations in the neurons of the dentate gyrus and cornu ammonis3 of the hippocampus of rats evidenced by alteration of the microanatomy of their granule and pyramidal neurons respectively. Concurrent administration of MSG with TOE and VIT E demonstrated amelioration of the behavioural changes and histological alterations of the hippocampal neurons.

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