 DOI: https://doi.org/10.82351/wajp.vol37no2.485

Antioxidant Effect of *Hibiscus sabdariffa* calyx Aqueous Extract on Mice Brains Exposed to Monosodium Glutamate- Induced Alterations

Adaeze N. Adebesin[1], Esther A. Ambali[1] and Abayomi M. Ajayi[2]

[1]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria.

Corresponding author: Esther A. Ambali
Email: ambaliesther11@gmail.com
Telephone: +2347034259650

ABSTRACT

Background: Monosodium glutamate (MSG), a common flavor enhancer, has been linked to neurotoxicity and oxidative stress in the brain, contributing to neurodegenerative changes. *Hibiscus sabdariffa* calyx aqueous extract (HSAE), rich in polyphenols and anthocyanins, possesses antioxidant properties that may counteract such effects. Objective: To evaluate the antioxidant and neuroprotective potential of HSAE against MSG-induced brain alterations in mice.

Method: Male mice were divided into six groups (n=6): control, MSG (2.5 g/kg), fluoxetine (20 mg/kg), and HSAE (50, 100, and 200 mg/kg) co-administered with MSG for 14 days respectively. Oxidative stress markers including malondialdehyde (MDA), nitrites, glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase were assessed, alongside histological examination of the dentate gyrus.

Result: MSG significantly increased MDA, nitrites, and acetylcholinesterase levels while decreasing GSH, CAT, and SOD, indicating oxidative damage and cholinergic dysfunction. HSAE (100 and 200 mg/kg) and fluoxetine significantly reversed these effects. Notably, HSAE (200 mg/kg) restored antioxidant enzyme levels and neuronal integrity comparable to fluoxetine.

Conclusion: HSAE significantly demonstrated strong antioxidant and neuroprotective effects against MSG-induced neurotoxicity, particularly at 200 mg/kg, by reducing oxidative stress and preserving brain structure.

Keywords: *Hibiscus sabdariffa calyx*, monosodium glutamate, oxidative stress, neuroprotection, dentate gyrus.

 DOI: https://doi.org/10.82351/wajp.vol37no2.485

Effet antioxydant de l'extrait aqueux de calices *d'Hibiscus sabdariffa* sur les cerveaux de souris exposées à des altérations induites par le glutamate monosodique

Adaeze N. Adebesin[1], Esther A. Ambali[1] and Abayomi M. Ajayi[2]

[1]Département de pharmacologie et de thérapeutique, Faculté des sciences médicales de base, Collège des sciences de la santé Obafemi Awolowo, Université Olabisi Onabanjo, Campus de Sagamu, État d'Ogun, Nigéria.
[2]Département de pharmacologie et de thérapeutique, Faculté des sciences médicales fondamentales, Université d'Ibadan, Ibadan, Nigéria.

Auteur correspondant : Esther A. Ambali
E-mail : ambaliesther11@gmail.com
Téléphone : +2347034259650

RÉSUMÉ

Contexte : Le glutamate monosodique (GMS), un exhausteur de goût couramment utilisé, a été associé à une neurotoxicité et à un stress oxydatif cérébral, contribuant ainsi à des altérations neurodégénératives. L'extrait aqueux de calice *d'Hibiscus sabdariffa* (EACHS), riche en polyphénols et en anthocyanes, possède des propriétés antioxydantes susceptibles de contrecarrer ces effets.

Objectif : Évaluer le potentiel antioxydant et neuroprotecteur de l'EACHS contre les altérations cérébrales induites par le GMS chez la souris.

Méthode : Des souris mâles ont été réparties en six groupes (n=6) : un groupe témoin, un groupe GMS (2,5 g/kg), un groupe fluoxétine (20 mg/kg) et un groupe EACHS (50, 100 et 200 mg/kg) co-administré avec du GMS, pendant 14 jours. Les marqueurs du stress oxydatif, notamment le malondialdéhyde (MDA), les nitrites, le glutathion (GSH), la catalase (CAT), la superoxyde dismutase (SOD) et l'acétylcholinestérase, ont été évalués, parallèlement à un examen histologique du gyrus denté.

Résultats : Le glutamate monosodique (GMS) a entraîné une augmentation significative des taux de MDA, de nitrites et d'acétylcholinestérase, tout en diminuant ceux de GSH, de CAT et de SOD, ce qui indique des lésions oxydatives et un dysfonctionnement cholinergique. L'extrait aqueux EACHS (100 et 200 mg/kg) et la fluoxétine ont significativement inversé ces effets. Notamment, l'extrait EACHS (200 mg/kg) a rétabli les taux d'enzymes antioxydantes et l'intégrité neuronale de manière comparable à la fluoxétine.

Conclusion : L'EACHS a démontré de manière significative de puissants effets antioxydants et neuroprotecteurs contre la neurotoxicité induite par le GMS, en particulier à la dose de 200 mg/kg, en réduisant le stress oxydatif et en préservant la structure cérébrale.

Mots-clés : calice *d'Hibiscus sabdariffa*, glutamate monosodique, stress oxydatif, neuroprotection, gyrus denté.

INTRODUCTION

Plants have been a vital source of medicine for humanity for thousands of years.[1] Different portions of plants, such as stems, leaves, flowers, roots, or seeds, are used in herbal medicine, also known as botanical medicine or phytomedicine, to treat and prevent diseases. Improved general health and well-being are common goals of using herbal remedies.[2] Medicinal herbs are plants with potential medicinal and health benefits. In recent years, they have been increasingly used as a treatment alternative owing to their effectiveness against various diseases.[3]

Herbal medicine is the study of pharmacognosy and the use of medicinal plants. Medicinal plants have played a crucial role as sources of ingredients for medications and drug synthesis. These plants contain bioactive compounds, including tannins, alkaloids, sugars, terpenoids, steroids, and flavonoids, with diverse therapeutic applications.[4] They are known to treat a variety of illnesses in both humans and animals. Notably, the leaves of these plants are the most frequently used part.[5]

Herbs and plants can be processed and can be taken in different ways and forms, and they include whole herbs, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract. Plants and herbs extract vary in the solvent used for extraction, temperature, and extraction time, and include alcoholic extracts (tinctures), vinegar (acetic acid extracts), hot water extract (tisanes), long-term boiled extract, usually roots or bark (decoctions), and cold infusion of plants (macerates).[6] Herbal medicines may have negative effects, including allergic reactions, asthma, headaches, nausea, vomiting, and diarrhea.

Hibiscus sabdariffa is a tropical plant known for its vibrant red calyxes, used for making wine, juice, jam, syrup, pudding, cakes, ice cream, or tea, and belongs to the genus *Hibiscus*.[7] Commonly referred to as Roselle or Sorrel, *Hibiscus sabdariffa* is an annual herbaceous shrub that belongs to the Malvaceae family. The extract of this plant has been found to have numerous bioactive properties, including antioxidant, anti-tumor, anti-hyperammonemia, anti-atherosclerotic, anti-filarial, and anti-hyperlipidemic activities. Additionally, the leaves of *H. sabdariffa* are rich in phenolic compounds, which are likely responsible for their antioxidant capacity.[8]

Roselle (*Hibiscus sabdariffa*) is recognized for its antioxidant, antimicrobial, anti-inflammatory, and anti-proliferative properties.[9] It is rich in phenolic compounds, which have anti-obesogenic and antidiabetic effects. The plant extract comes in two varieties: Alma Blanca, which has a white-yellow color, and Cuarenteña, which is purple and is the variety used in this project.[10]

Antioxidants are primarily found in food and medicinal plants, including fruits, vegetables, cereals, mushrooms, beverages, flowers, spices, and traditional medicinal herbs. The natural antioxidants sourced from these plant materials mainly consist of polyphenols (such as phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (including xanthophylls and carotenes), and vitamins (specifically vitamins E and C). Polyphenols and carotenoids, in particular, demonstrate a wide range of biological effects, including anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer properties.[11]

Natural antioxidants can protect the human body from free radicals and retard the lipid oxidative rancidity in foods. According to previous studies, *H. sabdariffa* has long been recognized as a source of antioxidants.8 Its petals are potentially a good source of antioxidant agents such as anthocyanins and ascorbic acid. The antioxidant activity of the Roselle extract correlated strongly with its anthocyanin content, which also was found to be very high in ascorbic acid content or ascorbate, which is a well-known natural antioxidant and excellent reducing agent. Reducing power is to measure the reductive ability of antioxidants, and it is evaluated by the transformation of Fe (III) to Fe (II). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) technique is mostly employed to assess the antioxidant activity of H. sabdariffa leaves and calyxes.[12]

The German chemist Karl Heinrich Ritthausen discovered monosodium glutamate. MSG is a subset of glutamate which is an important but "non-essential" amino acid found in several foods, including beef, milk, tuna, and vegetables. It plays an important role in human metabolism. MSG is formulated from water, glutamate, and sodium. Toxicities such as hepatotoxicity, genotoxicity, reproductive toxicity, and renal toxicity, as well as neurotoxic effects, have also been reported to accompany MSG intake. MSG ingestion has also been linked with Parkinson's disease, Alzheimer's disease, addiction, brain trauma, anxiety, stroke, depression, male reproductive dysfunction, and epilepsy.[13]

Monosodium glutamate (MSG), the sodium salt of glutamate is a widely used flavor enhancer that has raised concerns about its potential negative effects on various organs like the brain.[14] MSG also causes immunosuppression by disrupting redox equilibrium and inducing oxidative stress.[15]

However, no extensive literature research has been done previously on effect of HSAE on MSG-induced toxicity hence, the study sought to investigate the potential antioxidant properties of HSAE in mitigating oxidative stress and neuronal damage in the brains of mice subjected to alterations caused by MSG. Through this research, we seek to determine the extent to which HSAE can protect brain tissues from the harmful effects associated with MSG exposure, thereby contributing to a better understanding of its neuroprotective capabilities and potential therapeutic applications in oxidative stress-related conditions.

METHODS

Drugs and chemicals

Distilled water, fluoxetine (Bristol laboratories Ltd; United Kingdom), monosodium glutamate (MSG) (Vedan Seasoning Enterprises; Taiwan), *Hibiscus sabdariffa* calyx aqueous extract (HSAE), methylated spirit, and formalin, sterile needles and syringes, cotton wool, gloves. Cooler, animal cages, markers, paper tape, dissecting scissors, forceps, complete dissecting kits, and lithium heparinized bottles. All materials were handled according to standard laboratory protocols to ensure consistency, accuracy, and reproducibility of results.

Plant material and extraction preparation

Fresh, healthy calyces of *Hibiscus sabdariffa* were obtained from a local market in Ibadan, Oyo State, Nigeria, and the calyces were sorted to remove debris and damaged material, air-dried under shade at room temperature for fourteen days, and subsequently milled into a coarse powder using a mechanical grinder. The aqueous extract was prepared by macerating 500 g of the dried powdered calyx in 2.5 L of distilled water for 48 hours at room temperature with intermittent agitation. The macerate was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40ºC and subsequently freeze-dried to obtain a dry powder, designated *Hibiscus Sabdariffa* calyx aqueous extract (HSAE). The extract was stored in an airtight container at 4ºC until use and reconstituted in distilled water immediately before administration.

Experimental animals

Swiss male mice weighing 20-25 g was used in this experiment. The animals were obtained at an animal house in Sagamu near the institution, transported to the Pharmacology Laboratory at Olabisi Onabanjo University, Sagamu campus, where the study was conducted. Upon arrival, they were housed in cages under standard environmental conditions, including regulated light cycles and humidity. The animals were fed pelletized feed (Vital Feeds Ltd, PLC) and had free access to water throughout the study period. To minimize stress and ensure physiological stability, the mice were allowed to acclimatize for seven days prior to the commencement of the experiment.

Experimental design

Thirty-six mice were randomly divided into six groups (n = 6 per group). Group I served as the control group and received neither induction nor treatment. Group II received MSG alone at a dose of 2.5 g/kg. Groups III, IV, and V were treated with MSG in combination with HSAE at doses of 50 mg, 100 mg, and 200 mg, respectively. Group VI received MSG along with fluoxetine (20 mg/kg) as a standard reference drug.

All treatments were freshly prepared daily and administered once daily for 14 consecutive days. MSG was administered via the subcutaneous route, while HSAE and fluoxetine were administered intraperitoneally. Treatments were given 30 minutes prior to behavioral testing. After treatment administration, behavioral assessments were conducted, followed by sacrifice of the animals for brain tissue collection. The brain samples were homogenized, and the supernatants were separated and stored for subsequent antioxidant and biochemical assays.

Biochemical studies

Immediately after behavioral assessments, blood samples were collected from the retro-orbital sinus. The animals were then sacrificed via cervical dislocation, and whole brains were excised, harvested, and processed for further analysis. Brain tissues were homogenized, and the resulting supernatants were used for the estimation of oxidative stress and antioxidant parameters, including malondialdehyde (MDA), nitrite levels, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE) activities using spectrophotometric methods.

Determination of glutathione (GSH) concentration

The determination of reduced glutathione (GSH) serves as a reliable indicator of oxidative stress.[16] GSH levels in brain tissues were estimated using the method of Moron *et al*., (1979).[17] Following centrifugation of the brain homogenate, equal volumes of sample and 20 % trichloroacetic acid were mixed and centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was then reacted with DTNB and phosphate buffer to produce a measurable color change. Absorbance was read at 412 nm against a blank using a spectrophotometer. GSH values were expressed in micromoles per gram of tissue (µmol/g tissue).[18]

Effect on lipid peroxidation

Lipid peroxidation was evaluated by measuring MDA levels using the method of Adam-Vizi and Seregi (1982).[19] An aliquot of the sample was mixed with Tris-KCl buffer, followed by the addition of trichloroacetic acid and thiobarbituric acid. The mixture was heated in a water bath at 80°C for 45 minutes, cooled, and centrifuged. The absorbance of the supernatant was measured at 532 nm against a blank. MDA concentration was calculated using a molar extinction coefficient of 1.56 × 10? M?¹ cm?¹ and expressed as nanomoles per milligram protein.[18]

Effect on superoxide dismutase (SOD) activity

SOD activity was determined using the method of Misra and Fridovich.[20] Brain samples were added to carbonate buffer and equilibrated in a spectrophotometer. The reaction was initiated by adding adrenaline, and absorbance changes were monitored at 480 nm at defined intervals. The reference cuvette contained buffer, adrenaline, and distilled water. SOD activity was calculated based on the inhibition of adrenaline autoxidation and expressed as micromoles per milligram protein (µmol/mg protein).

Estimation of brain nitrite concentration

Brain nitrite concentration, an indicator of nitric oxide production, was determined using the Griess reagent method (Green *et al*., 1982).[21] Equal volumes of brain supernatant and Griess reagent were mixed, and absorbance was measured at 540 nm. Nitrite levels were calculated from a sodium nitrite standard curve (0-100 µM) and expressed as micromoles per milligram protein (µmol/mg protein).

Effect on catalase (CAT) activity

Catalase activity was measured using a colorimetric assay based on the formation of a yellow complex between ammonium molybdate and hydrogen peroxide, as described by Goth (1991).[22] The absorbance was read at 405 nm using a UV/Vis spectrophotometer, and enzyme activity was expressed as kilo units per milligram protein (kU/mg protein) in brain tissue samples.

Effect on acetylcholinesterase (AChE) activity

Acetylcholinesterase activity was determined using the method of Ellman *et al*., (1961).[23] Brain supernatant was mixed with phosphate buffer and DTNB, followed by the addition of acetylthiocholine iodide. The reaction produced a colored complex, and absorbance changes were monitored at 412 nm at regular intervals. The rate of change in absorbance per minute was used to calculate enzyme activity, which was expressed as µmol/min/mg protein.

Assessment of histological alterations in mice brains

Brain tissues were fixed in 10 % phosphate-buffered formaldehyde and processed into paraffin-embedded blocks. Sections were cut in the sagittal plane using a microtome and stained with hematoxylin and eosin. The stained sections were examined under a binocular research microscope, and images were captured to assess histological changes, particularly in the hippocampal dentate gyrus region.

Statistical evaluation

Data obtained from the study were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software (version 8.5). Differences between experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was considered at $P < 0.05$.

RESULTS

Effect of hibiscus sabdariffa calyx aqueous extract (HSAE) on concentration of malondialdehyde and nitrites in brains of mice administered with monosodium glutamate (msg).

The effect of HSAE on the concentration of malondialdehyde (MDA) and nitrites in mice exposed to MSG is shown. Elevated brain levels of MDA and nitrites concentration induced by MSG administration were significantly reversed by HSAE (100 and 200 mg/kg, i.p) in mice. Also, similar effects were observed in the group that were given the standard fluoxetine (Flx) (20 mg/kg, i.p) although not significantly different (figure 1&2).

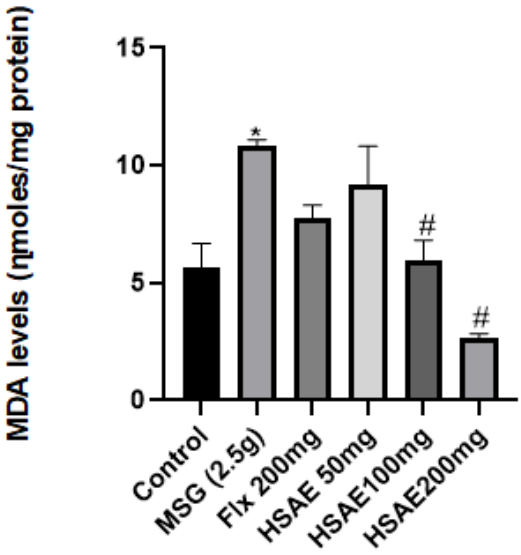


Figure 1: Effect of HSAE on MDA concentration in monosodium glutamate (MSG)-exposed mice. Vertical bars represent mean ± S.E.M for 6 animals per group. *p < 0.05 when compared with control and #p < 0.05 when compared to MSG (ANOVA followed by Turkey post hoc test).

MSG- monosodium glutamate, Flx- flouxetine, HSAE- *Hibiscus sabdariffa* calyx aqueous extract.

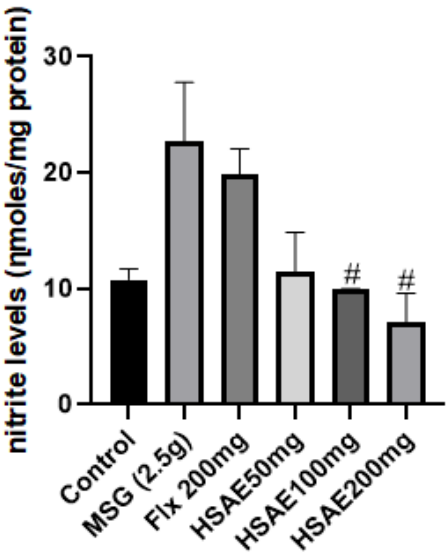


Figure 2: Effect of HSAE on nitrites concentration in monosodium glutamate (MSG)-exposed mice. Vertical bars represent mean ± S.E.M for 6 animals per group. *p < 0.05 when compared with control and #p < 0.05 when compared to MSG (ANOVA followed by Turkey post hoc test).

MSG- monosodium glutamate, Flx- flouxetine, HSAE- *Hibiscus sabdariffa* calyx aqueous extract.

Effect of *hibiscus sabdariffa* calyx aqueous extract (HSAE) on the levels of acetylcholinesterase in brains of mice administered with monosodium glutamate (msg).

The effect of HSAE on the levels of acetylcholinesterase in mice exposed to MSG is shown. Elevated brain levels of acetylcholinesterase induced by MSG administration were significantly reversed by HSAE (100 and 200 mg/kg,i.p) in mice. Also, similar effects were observed in the group given the standard fluoxetine (Flx) (20 mg/kg, i.p) not significantly different (figure 3).

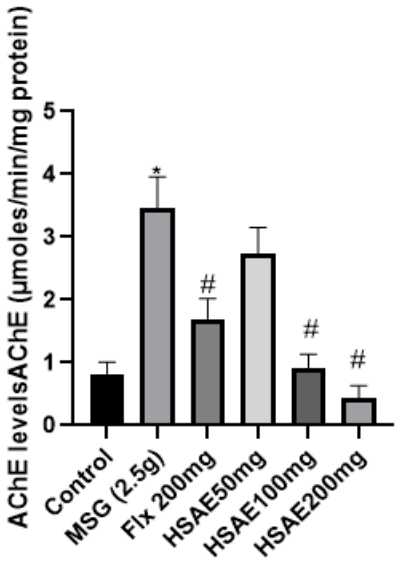


Figure 3: Effect of HSAE on the levels of acetylcholinesterase in monosodium glutamate (MSG)-exposed mice. Vertical bars represent mean ± S.E.M for 6 animals per group. *p < 0.05 when compared with control and #p < 0.05 when compared to MSG (ANOVA followed by Turkey post hoc test).

MSG- monosodium glutamate, Flx- flouxetine, HSAE-*Hibiscus sabdariffa* calyx aqueous extract.

Effect of *hibiscus sabdariffa* calyx aqueous extract (HSAE) on monosodium glutamate (msg)-alteration in levels of antioxidants in mice brain.

The effect of HSAE on the concentration of glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in mice exposed to MSG is shown. Decreased brain levels of GSH, SOD and CAT concentration induced by MSG administration were increased by HSAE and flouxetine in mice, with the HSAE 200mg/kg and flx group alone showing significant increase in the GSH concentration (Table 1).

Table 1: Effect of HSAE treatment on the antioxidant parameters in brains of mice after exposure to MSG.

Treatment	GSH Concentration (µmol/g tissue)	CAT Levels (units/mg protein)	SOD Levels (units/mg protein)
Control	49.93 ± 0.88	60.12 ± 6.67	3.75 ± 0.82
MSG (2.5 g/kg)	31.15 ± 0.80*	49.22 ± 1.50	2.82 ± 0.53
Fluoxetine (20 mg/kg)	63.01 ± 3.07#	92.91 ± 13.08#	4.84 ± 0.72
HSAE (50 mg/kg)	37.51 ± 1.44	86.73 ± 15.91	3.74 ± 0.86
HSAE (100 mg/kg)	43.82 ± 3.75	64.30 ± 2.98	3.27 ± 0.26
HSAE (200 mg/kg)	55.86 ± 2.92#	88.53 ± 1.08	3.52 ± 0.73

*p < 0.05 when compared with control and #p < 0.05 when compared to MSG (ANOVA followed by Turkey post hoc test).

Effects of HSAE on histological alterations of the dentate gyrus of mice brains subjected to msg treatment.

The effect of HSAE treatment and MSG exposure on neuronal cells in mice brain. Control shows a well-defined and normal morphology and organization while the MSG group shows some disruption in cell organization and reduced granule cell density with mild signs of degeneration. The HSAE treatment groups show some slight restoration with the HSAE 200 mg/kg group showing a better preservation of the dentate gyrus (DG) compared to the lower doses and compared with MSG group. A similar restorative effect was observed in the flouxetine group plus a preserved architecture (Figure 4)

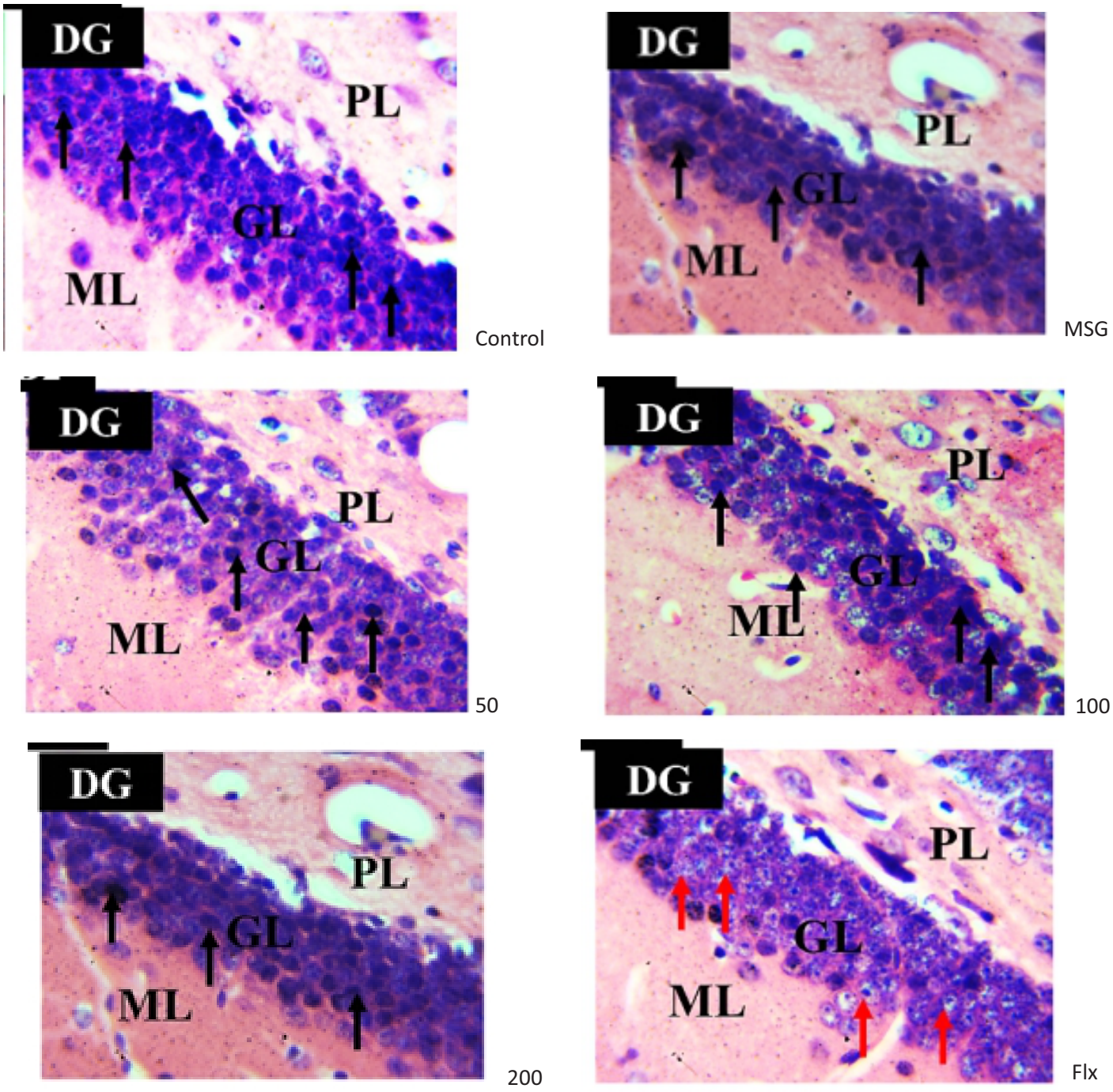


Figure 4: Photomicrograph of the representative sections of the dentate gyrus (DG) of mice brains treated with MSG (2.5 g/kg), HSAE (50, 100, 200 mg/kg) and Flx (20 mg/kg). DG-dentate gyrus, PL- polymorphic layer, GL- granule cell layer, ML- molecular layer.

DISCUSSION

This study investigated the neuroprotective effects of *Hibiscus sabdariffa* calyx aqueous extract (HSAE) against monosodium glutamate (MSG)-induced oxidative stress and neurotoxicity in mice brains. The findings demonstrated that MSG exposure significantly disrupted the oxidative balance in the brain, as evidenced by increased levels of malondialdehyde (MDA) and nitrites, alongside a marked reduction in endogenous antioxidant defenses, including reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). These observations are consistent with previous studies showing that MSG induces the generation of reactive oxygen species (ROS), leading to oxidative damage and neuronal dysfunction.[24-25] In addition to biochemical alterations, this study also assessed histomorphological changes in the hippocampal dentate gyrus (DG), further elucidating the structural consequences of MSG toxicity and the protective role of HSAE.

The elevation of MDA and nitrite levels observed in MSG-treated mice reflects enhanced lipid peroxidation and nitric oxide production, both of which are hallmarks of oxidative and nitro-active stress. However, administration of HSAE at doses of 100 and 200 mg/kg significantly reversed these increases, indicating its capacity to mitigate oxidative damage. A similar trend was observed with the standard reference drug fluoxetine (Flx), although the differences between HSAE and fluoxetine-treated groups were not statistically significant. These findings corroborate earlier reports that MSG elevates MDA and nitrite concentrations through oxido-nitrergic mechanisms involving glutamate excitotoxicity.[26] The ability of HSAE to reduce these markers supports its antioxidant potential, likely attributable to its rich composition of bioactive compounds such as anthocyanins, flavonoids, and polyphenols, which have been widely reported to scavenge free radicals and inhibit oxidative processes.[27-28]

Another important finding of this study is the modulation of acetylcholinesterase activity. MSG administration resulted in elevated acetylcholinesterase levels, which may reflect impaired cholinergic neurotransmission and neuronal dysfunction. Treatment with HSAE significantly reduced acetylcholinesterase activity at both 100 and 200 mg/kg doses, suggesting restoration of cholinergic balance. Fluoxetine produced comparable effects, further supporting the neuroprotective role of HSAE. These findings are consistent with the study by Gad *et al*.,[29] which demonstrated that HSAE reduced oxidative stress and improved histological outcomes in MSG-induced testicular damage. Although that study focused on non-neural tissue, the underlying antioxidant and antiapoptotic mechanisms-such as reduction of ROS and enhancement of antioxidant enzyme activity-are likely applicable to brain tissue. Therefore, HSAE may exert its neuroprotective effects through similar pathways, including modulation of neurotransmitter-related enzymes and attenuation of oxidative stress.

The depletion of antioxidant enzymes following MSG exposure highlights the vulnerability of neural tissues to oxidative damage. In this study, HSAE effectively restored the levels of GSH, CAT, and SOD, particularly at higher doses. The restoration of these enzymes suggests improved cellular defense mechanisms against ROS. These results are in agreement with previous findings demonstrating the antioxidant efficacy of Hibiscus sabdariffa extracts. For instance, Farag & Osman in 2009, reported that HSAE significantly reduced lipid peroxidation and restored antioxidant enzyme activity in MSG-treated rats.[30] The beneficial effects were attributed to the presence of phenolic compounds and anthocyanins, which enhance the body's antioxidant capacity.

Specifically, the restoration of GSH levels observed in this study indicates improved intracellular redox balance. MSG-induced depletion of GSH is a well-established indicator of oxidative stress, and its recovery following HSAE treatment underscores the extract's protective role. Similar findings have been reported by Ezejiofor *et al*.,[31] who showed that HSAE restored GSH levels in MSG-induced liver damage. Additionally, Mahmoud *et al*.,[32] demonstrated the ability of HSAE to recover GSH levels in gentamicin-induced nephrotoxicity. The increase in CAT activity observed in this study further supports enhanced detoxification of hydrogen peroxide, a key reactive species. The moderate improvement in SOD activity is consistent with the findings of Ngulde *et al*.,[33] indicating that HSAE contributes to the dismutation of superoxide radicals and overall oxidative balance.

Histological evaluation of the hippocampus provided additional insights into the neuroprotective effects of HSAE. The control group exhibited normal cytoarchitecture, with well-organized layers including the granular layer (GL), molecular layer (ML), and polymorphic layer (PL), and densely packed granule cells. In contrast, MSG-treated mice showed significant histopathological alterations, including reduced cell density, disrupted

layering, vacuolation, and neuronal loss. These features are indicative of glutamate-induced excitotoxicity, a process characterized by excessive activation of glutamate receptors, increased intracellular calcium influx, oxidative stress, and eventual neuronal death.[34]

Co-administration of HSAE with MSG resulted in notable improvements in hippocampal structure in a dose-dependent manner. At 50 mg/kg, partial restoration of the dentate gyrus was observed, with reduced cellular damage. The 100 mg/kg dose produced more pronounced improvements, including increased granule cell density and reduced vacuolation. Remarkably, the 200 mg/kg dose demonstrated substantial preservation of hippocampal architecture, closely resembling that of the control group. These findings suggest that higher doses of HSAE confer greater neuroprotective benefits, likely due to enhanced antioxidant and anti-inflammatory effects. The preservation of neuronal integrity observed in HSAE-treated groups is consistent with the biochemical findings and reinforces the extract's role in preventing neurodegeneration.

The neuroprotective effects of HSAE may be attributed to its rich phytochemical composition, particularly polyphenols and anthocyanins, which possess strong antioxidant and anti-inflammatory properties.[35] These compounds likely contribute to the stabilization of cellular membranes, inhibition of lipid peroxidation, and modulation of signaling pathways involved in inflammation and cell survival. In comparison, fluoxetine, used as a standard reference drug, also demonstrated significant neuroprotective effects. Fluoxetine is known to exert its actions through modulation of serotonin levels and upregulation of brain-derived neurotrophic factor (BDNF), thereby promoting neurogenesis and synaptic plasticity.[36] Additionally, increased serotonin levels have been linked to enhanced BDNF expression, supporting neuronal survival and functional recovery.[37]

Taken together, these results indicate that HSAE can ameliorate MSG-induced structural damage in the hippocampal dentate gyrus, with efficacy comparable to that of fluoxetine at higher doses, supporting its potential as a natural therapeutic agent against neurodegeneration.

CONCLUSION

Increased lipid peroxidation, nitro-active stress, AChE activity, and histological damage in mice brains of treated groups are evidence that MSG causes oxidative stress and neurotoxicity. *Hibiscus sabdariffa* calyx aqueous extract (HSAE), particularly at 200 mg/kg demonstrated its antioxidative and neuroprotective properties. HSAE can minimize MSG-induced neurotoxicity such as by lowering oxidative stress, boosting antioxidant defense, and preventing cholinergic disruption. It may also be used to treat oxidative stress-related neurodegenerative diseases. However, side effects for chronic and repeated treatment of the HSAE and MSG needs to be investigated in the future.

ETHICAL APPROVAL

Ethical approval number (UI-ACUREC/087-0923/22) was assigned from the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC), and the experiments complied with the WMA Statement on animal use in biomedical research.

REFERENCES

1. Davis CC, Choisy P (2024). Medicinal plants meet modern biodiversity science. *Current Biology* 34: R158-R173.
2. Ingale S, Rathored J, Wankhade S, Nibude S. (2025). Exploring the use of herbal medicines in the context of dermatokinetic research. *Journal of Young Pharmacists* 17: 19-25.
3. Takada K, Nakano S, Nishio R, Muku D, Mochizuki S, Inui I, Okita K, Koga A, Watanabe K, Yoshioka Y, Ariyoshi W, Yamasaki R (2024). Medicinal herbs, especially *Hibiscus sabdariffa*, inhibit oral pathogenic bacteria. *Journal of Oral Biosciences* 66: 179-187.
4. Aliya F, Yunus MH, Izhar SK, Afaq U (2025). Medicinal plants in the treatment of respiratory diseases and their future aspects. *Recent Patents on Biotechnology* 19: 2-18.
5. Nuro G, Tolossa K, Arage M, Giday M (2024). Medicinal plants diversity among the Oromo community in Heban-Arsi district of Ethiopia used to manage human and livestock ailments. *Frontiers in Pharmacology* 15: 1455126.
6. Wachtel-Galor S, Benzie IFF (2011). Herbal medicine: An introduction to its history, usage, regulation, current trends, and research needs. In I. F. F. Benzie & S. Wachtel-Galor (Eds.), Herbal medicine: *Biomolecular and clinical aspects*; CRC Press.
7. Espinosa-Sánchez A, Montaño-Estrada LF, Rendón-Huerta EP, Navarrete-Peón A, Gómez-Aldapa CA, Muñóz-Pérez VM, Castro-Rosas J. (2024), *Hibiscus sabdariffa* as a Novel Alternative Strategy Against

Helicobacter pylori Infection Development to Gastric Cancer. *Journal of Medicinal Food* 27(12):1158-1167.

8. Wang J, Cao X, Jiang H, Qi Y, Chin KL, Yue Y (2014). Antioxidant activity of leaf extracts from different *Hibiscus sabdariffa* accessions and simultaneous determination of five major antioxidant compounds by LC-Q-TOF-MS. Molecules, 19, 21226-21238.
9. Bernardes AL, Moraes LFS, Cruz BCDS, da Conceição LL, de Oliveira LL, Sarandy MM, Gonçalves RV, Peluzio MDCG (2023). *Hibiscus sabdariffa* supplementation increases butyrate synthesis and reduces inflammatory cells, attenuating the formation of aberrant crypt foci in BALB/c mice induced to pre-neoplastic lesions. *British Journal of Nutrition* 129: 352-363.
10. Rangel-García CA, Reynoso-Camacho R, Pérez-Ramírez IF, Morales-Luna E, de Los Ríos EA, Salgado LM (2022). Serum phospholipids are potential therapeutic targets of aqueous extracts of roselle (*Hibiscus sabdariffa*) against obesity and insulin resistance. International *Journal of Environmental Research and Public Health* 19: 16538.
11. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *International Journal of Molecular Sciences*, 18: 96.
12. Ngondo B, Mpika J, Mbon N (2023). Antioxidant activity of phenolic compounds in *Hibiscus sabdariffa* from Congo. *African Journal of Agricultural Research*. 19: 1056-1068.
13. Kayode OT, Rotimi DE, Kayode AAA, Olaolu TD, Adeyemi OS (2020). Monosodium glutamate (MSG)-induced male reproductive dysfunction: A mini review. *Toxics* 8: 7.
14. Gbadamosi I, Yawson EO, Akesinro J, Adeleke O, Tokunbo O, Bamisi O, Ibrahim-Abdulkareem R, Awoniran P, Gbadamosi R, Lambe E, Atoyebi A, Bayo-Olugbami AA, Abayomi TA, Arogundade TT (2024). Vitamin D attenuates monosodium glutamate-induced behavioural anomalies, metabolic dysregulation, cholinergic impairment, oxidative stress, and astrogliosis in rats. *Neurotoxicology*, 103: 297-309.
15. Das D, Banerjee A, Manna K, Sarkar D, Shil A, Sikdar N, Bhakta M, Mukherjee S, Maji BK. (2023). Quercetin counteracts monosodium glutamate to mitigate immunosuppression in the thymus and spleen via redox-guided cellular signaling. *Phytomedicine* 126: 155226.
16. Nuhu F, Gordon A, Sturmey R, Seymour AM, Bhandari S (2020). Measurement of glutathione as a tool for oxidative stress studies by high performance liquid chromatography. *Molecules* 25: 4196. https://doi.org/10.3390/molecules25184196.
17. Moron MS, DePierre JW, Mannervik B (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta* 582: 67-78.
18. Adeoluwa OA, Agboola I, Akinluyi E, Eduviere A, Adeoluwa AG, Ajayi A, Aderibigbe A (2023). N-butanol fraction of Olax subscorpioidea attenuates lipopolysaccharide-induced depressive-like symptoms in mice. *Research Square*, 7: 19.
19. Adam-Vizi V, Seregi M (1982). Receptor independent stimulatory effect of noradrenaline on Na^+/K^+-ATPase in rat brain homogenate: Role of lipid peroxidation. *Journal of Neurochemistry* 38: 1148-1152.
20. Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry* 247: 3170-3175.
21. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical Biochemistry*, 126: 131-138.
22. Góth L (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta* 196: 143-151.
23. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7: 88-95.
24. Eweka AO, Om'Iniabohs FAE (2007). Histological studies of the effects of monosodium glutamate on the small intestine of adult Wistar rats. *Internet Journal of Gastroenterology* 6(2).
25. Farombi EO, Onyema OO (2006). Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: Modulatory role of vitamin C, vitamin E and quercetin. *Human & Experimental Toxicology* 25: 251-259.
26. Omogbiya AI, Ben-Azu B, Eduviere AT, Eneni AO, Nwokoye PO, Ajayi AM, Umukoro S (2020). Monosodium glutamate induces memory and hepatic dysfunctions in mice: Ameliorative role of Jobelyn®. *Toxicology Research* 37: 323-335.
27. Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003). Analysis and biological activities of anthocyanins. *Phytochemistry* 64: 923-933.

28. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M (2014). *Hibiscus sabdariffa* L.-A phytochemical and pharmacological review. *Food Chemistry* 165: 424-433.
29. Gad SB, Zaghloul DM, Abdel-Rahman GH (2020). Protective effect of Hibiscus sabdariffa extract against monosodium glutamate-induced testicular toxicity in rats: Role of oxidative stress and apoptosis. *Journal of Complementary and Integrative Medicine* 17: 20190135.
30. Farag MF, Osman N (2009). Antioxidant Actions of Irradiated Hibiscus sabdariffa L. (KARKADE) Against Monosodium Glutamate-Induced Oxidative Stress in Rats. *Isotope and Radiation Research*, 41.
31. Ezejiofor AN, Orisakwe OE, Obi E (2020). Antioxidant and hepatoprotective effects of *Hibiscus sabdariffa* aqueous extract against MSG-induced liver damage. *Journal of Applied Pharmaceutical Science* 10: 120-127.
32. Mahmoud RH, Mohamad HF (2014). *Hibiscus sabdariffa* L. attenuates nephrotoxicity and oxidative stress. *American Journal of Food Technology* 9(6): 299-307.
33. Ngulde SS, Usman H, Yahaya Y (2021). Antioxidant and neuroprotective effect of *Hibiscus sabdariffa* against MSG-induced brain damage in rats. *Heliyon*, 7(1).
34. Choi DW (2020). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, 106: 866-887.
35. Arias-Sánchez S, Vázquez-Medina JP, Fernández-García E (2023). Phytochemical composition and biological activities of Hibiscus sabdariffa L.: A review of its antioxidant and anti-inflammatory properties. *Food Chemistry* 405: 134824.
36. Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nature Medicine* 22: 238-249.
37. Kitahara Y, Tsurugizawa T, Uematsu A, Ogawa M, Kato F, Saito Y (2016). Chronic fluoxetine treatment increases hippocampal neurogenesis and brain-derived neurotrophic factor levels in adult mice. *Neuroscience Research* 110: 56-63.