EVALUATION OF *INVITRO* AND *INVIVO* ANTIOXIDANTS ACTIVITIES OF MONOSODIUM GLUTAMATE

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Abstract: Monosodium glutamate (MSG), derivative of glutamic acid; food additive that enhance flavour, were associated with oxidative stress (potential health risks). Glutamate naturally occurs in high-protein foods, excessive consumption of MSG has raised concerns. This study evaluated the *in- vitro* and *in-vivo* antioxidant activities of monosodium MSG to ascertain the controversies surrounding its usage as a food additive. Acclimatized male Sprague-Dawley rats (n=40) were randomly selected and grouped into four: Control (A), 3% MSG (500 mg/kg) (B), 12% MSG (2000 mg/kg) (C), and 24% MSG (4000 mg/kg) (D). They were orally administered for 60 days. After the experimental period, blood samples were collected from the animals from orbital sinus. In-vitro antioxidant activity of MSG was determined by Evaluating: DPPH, FRAP, NO, OH, and LPO scavenging activities and they compared with standard (garlic acid/catechin). In-vivo antioxidant activities were determined from blood serum. The Result showed that, Lipid peroxidation marker (MDA), oxidative stress marker enzymes: SOD, GSH, and CAT. DPPH, FRAP, NO, OH and LPO scavenging activities showed that there was significant reduction in percentage inhibition produced by MSG when compared to the standard (garlic acid/ catechin) used. There were increase in CAT levels in group B and C but a decrease in group D, and a general increase in SOD levels in all the groups. GSH level showed a significant increase in group B while in groups C and D there was a non- significant decrease. The MDA levels showed decrease in group B and increase in groups C and D. MSG from the results of this study, can be said to have some potential antioxidant property. CAT. DPPH, FRAP, NO, OH and LPO scavenging activities showed that there is significant reduction ($p<0.05$) in percentage inhibition produced by MSG compared to the standard (garlic acid/catechin) used. There were increase CAT levels in group B and C but a decrease in group D, and a general increase in SOD levels in all the groups. GSH levels, showed a significant ($P<0.05$) increase in group B while groups C and D indicate a non-significant decrease. The MDA levels decrease in group B and increase in groups C and D. MSG, from the results of this study, can be said to have some potential antioxidant property. This study further confirmed, the uses of MSG between 500 mg/kg and 4000 mg/kg (below LD_{50}) for 60 days does not exhibit disruption in oxidative stress and inflammatory response.

Keywords: monosodium glutamate, in-vivo, in-vitro, antioxidant-properties.

Introduction

Oxidative stress, resulting from the presence of free radicals, is known to contribute to various human and animal diseases (Sies and Jones, 2020). Free radicals are unstable atoms or molecules that can cause damage to cellular components, including proteins, lipids, and DNA (Rahman et al., 2015). To counteract the harmful effects of free radicals, biological systems possess antioxidant defence mechanisms. However, disruptions in these protective mechanisms can occur due to both endogenous and exogenous factors, leading to oxidative damage (Sies and Jones, 2020).

Monosodium glutamate (MSG), a widely used food additive, has been associated with oxidative stress and potential health risks (Tsatsakis et al., 2019). MSG, derived from glutamic acid, is commonly used to enhance the flavour of various foods, particularly in Chinese, Japanese, and West African diets (Augustine et al., 2019; Bera et al., 2017). While glutamate naturally occurs in high-protein foods, excessive consumption of MSG has raised concerns regarding its potential harmful effects (Abdel-Reheim et al., 2014; Minal et al., 2023).

Numerous reports have linked MSG consumption to symptoms such as numbness, weakness, dizziness, flushing, and headaches, commonly known as the Chinese restaurant syndrome[7]. Furthermore, MSG has been associated with brain cell damage, retinal degeneration, endocrine disorders, and various pathological conditions, including stroke and epilepsy (Tawfik et al., 2012). Studies have also indicated negative effects on glucose metabolism, memory retention, hypothalamic damage, and mitochondrial function following excessive MSG intake (Hamza et al., 2014). Additionally, MSG has been implicated in the development of neurodegenerative disorders, obesity, infertility, growth retardation, and other health problems (Zanfirescu et al., 2019; Docea et al., 2018).

Evaluating the impact of MSG on oxidative stress and the antioxidant defence system is crucial for understanding its potential risks and developing strategies to mitigate its harmful effects. Therefore, this research study will give insight to the effects of MSG on physiological processes, oxidative stress, and the antioxidant defence system. This may shed light on the mechanisms underlying MSG-induced oxidative damage, and will contribute to our understanding of the risks associated with MSG consumption and the need for further investigations in this area. Moreover, this research aims to evaluate the in vitro and in vivo antioxidant activities of MSG, providing valuable insights into its potential role in modulating oxidative stress.

Methodology

Reagent and chemical

Monosodium Glutamate (Ajinomoto Co INC Tokyo, Japan), phosphate buffer (pH 7.4, 0.1 M) (Oxford laboratories, Mumbai India), bluing solution, glacial acetic acid, hydrochloric acid (HCl), Hellman reagent, glutathione acid, thiobarbituric acid, carbonate buffer, analytical glucose (Oxford laboratories, Mumbai India), Methylated sprit, sulphuric acid (H_2SO_4), naphthylenediamine dichloride, sulphanilic acid, ammonia solution, olive oil, ferric chloride ($FeCl_2$), Griess reagent, Sodium nitroprusside (Sigma-Aldrich, United States of America), trichloroacetic acid (TCA), potassium hexa-cyanoferrate (Guangdong Guanghua Sci-Tech Co., Ltd, China), Ascorbic acid, ammonium molybdate, sodium phosphate, potassium acetate, Aluminum chloride, Methanol, sodium carbonate solution (7.5% w/v) (BDH Laboratory Supplies, England), Folin- Ciocalteu reagent, potassium ferricyanide, Dragendroff's reagent, Mayer's reagent (Karl Fischer Reagents, UK), and Distilled water (from Manesty Distiller). All Chemicals used for this experiment were of analytical grade.

Ethical approval

This experiment was conducted in accordance to the rules and regulations of National Institute of Health[12] for laboratory animal care and use. Ethical clearance was obtained from the Olabisi Onabanjo University Teaching Hospitals Health Research Ethics Committee (OOUTH-HREC) with approval number OOUTH/HREC/669/2023AP. All the animal carcasses were buried deep in the ground covered with lime and disinfectant at least two feet beneath the natural surface and covered with soil.

Preparation of Monosodium Glutamate

The doses of monosodium glutamate used for this study was determined from the LD_{50} of 16,600mg/kg[13]. 10 g of monosodium glutamate was dissolved in 100 ml of distilled water to form the stock solution. The rats were administered with doses of 500 mg/kg, 2000 mg/kg and 4000 mg/kg from the stock solution according to the method described by Erhirhie (Erhirhie et al., 2014) using the formula:

$$\frac{\text{Dose rate x Body weight (kg)}}{\text{Stock concentration}}$$

Experimental Design and Treatment

In this experiment, we utilized forty healthy adult male Sprague-Dawley rats, each weighing between 70g and 100g. The rats were given a period of fourteen days to acclimate to their surroundings, during which they were provided with a standardized pellet diet and unrestricted access to water. After the acclimatization period, the rats were weighed and randomly divided into four groups, each consisting of ten rats. Group A, serving as the normal control, received only water. Group B, exposed to 3% MSG received a daily dosage of 500 mg/kg of MSG via oral gavage. Group C, subjected to 12% LD_{50}, received a daily dosage of 2000 mg/kg of MSG through oral gavage. Lastly, Group D, exposed to 24% LD_{50}, received a daily dosage of 4000 mg/kg of MSG via oral gavage. Throughout the experiment, the rats were housed in the animal house at Obafemi Awolowo College of Health Sciences in Sagamu, Ogun State, Nigeria. They were maintained under available atmospheric conditions. During both the acclimatization period and the experimental period, the rats had unrestricted access to the pellet diet and water, which were provided ad libitum. The administration of MSG was carried out daily for a total duration of sixty days. We administered MSG through the oral route using an oral cannula.

Animal Sacrifice and Collection of Blood Samples

Six (6) hours after the treatment period of sixty (60) days, the rats were placed in a closed jar containing cotton wool soaked with diethyl ether anesthetic. Following anesthesia, blood samples were collected from the retro orbital sinus, with the rats' neck gently scuffed and the eye made to bulge. A capillary tube was inserted dorsally into the eye and blood was allowed to flow by capillary action through the capillary tube. This was collected into the sample bottles.

Biochemical Analysis: Determination of Antioxidant Activities In-vitro Test

The radical scavenging activity of monosodium glutamate (MSG) was evaluated using stable radical, 2, 2-diphenyl-1-picrylhydrazyl according to the method described by Liyana and Shahidi (Liyana-Pathirana &Shahidi ,2005). The change in colour from deep violet/purple to light yellow was measured spectrophotometrically at 517 nm. The nitric oxide radical scavenging activity of MSG was determined adopting the method described by Oyedemi *et al* (Oyedemi et al., 2010). The reducing power of MSG was determined by the method described by Otang *et al.*, (Otang et al., 2012). Hydroxyl radical scavenging activity of the MSG was determined by the method of Halliwell *et al*.,(Halliwell et al., 1989). The thiobarbituric acid (TBA) assay was used to assess lipid peroxidation inhibition activities using the method of Bar-Or et al.,(Bera et al., 2012)

In-vivo Tests: SOD, CAT, GSH, MDA

SOD activity in the serum of the rats was determined by the method of Misra and Fridovich (Misra et al., 1972) Serum catalase activity was determined according to the method of Sinha. The total sulphydryl groups, protein-bound sulphydryl groups, free sulphydryl groups (such as reduced glutathione) in biological samples were determined using Ellman's reagent (DTNB) as described by Sedlak and Lindsay. Malondialdehyde activity (lipid peroxidation inhibition assay) in the rat serum was determined using a modified thiobarbituric acid reactive species (TBARS) assay described by Murugan and Parimelazhagan.

Statistical Analysis

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using Graph Pad Prism version 5 for Windows. Differences between groups were analyzed by one-way ANOVA followed by Dunnet post-hoc test. Differences were considered significant when $P < 0.05$.

Results and discussion

Results

In-vitro Tests

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) Free Radical Scavenging Activity Assay

The results obtained from this study regarding the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity revealed a significant reduction ($p<0.05$) in the percentage inhibition produced by MSG compared to the standard (garlic acid) that was used. The DPPH assay demonstrated that MSG possesses radical scavenging activity, and this activity increases with higher

concentrations, as shown in Table 1. However, it is important to note that the DPPH free radical scavenging effects of MSG were considerably lower compared to those of garlic acid at all corresponding concentrations. The results indicated an increase ranging from 0.45 at 20ug/ml to 28.16 at 100ug/ml showing an improvement in scavenging activity with increasing concentration. Nevertheless, it is evident that MSG exhibits lower activity compared to the standard (garlic acid).

Table 1: DPPH free radical scavenging activities of monosodium glutamate and garlic acid

Concentration (ug/ml)	Standard (Garlic Acid)	MSG
20	88.32 ± 0.97	0.45 ± 0.05*
40	88.87 ± 0.50	7.64 ± 0.34*
60	92.25 ± 1.01	15.95 ± 0.32*
80	93.49 ± 1.45	25.25 ± 0.66*
100	95.43 ± 2.06	28.16 ± 0.00*

Values are expressed as mean ± SEM of the different five concentrations.
***Significantly different from the standard at P< 0.05.**

Ferric reducing antioxidant power (FRAP) of monosodium glutamate and garlic acid

In the FRAP assay conducted for the range of concentrations examined (as shown in Table 2). Both monosodium glutamate (MSG) and the standard (garlic acid) exhibited concentration-dependent activity. However, it is important to note that there was a significant reduction ($p<0.05$) in the FRAP assay results when compared to the standard (garlic acid) that was used. The result indicated an increase ranging from 3.11 at 20ug/ml to 10.12 at 100ug/ml.

Table 2: Ferric reducing antioxidant power (FRAP) of monosodium glutamate and garlic acid.

Concentration (ug/ml)	Standard (Garlic Acid)	MSG
20	24.41 ± 0.55	3.11 ± 0.31*
40	43.62 ± 1.57	5.18 ± 0.11*
60	53.54 ± 1.33	6.97 ± 0.17*
80	86.88 ± 3.17	8.08 ± 0.07*
100	102.33 ± 0.56	10.12 ± 0.44*

Values are expressed as mean ± SEM of the different five concentrations.
***Significantly different from the standard at P< 0.05.**

Nitric oxide (NO) scavenging activities of monosodium glutamate and garlic acid

Table 3 presents the results of the nitric oxide scavenging activity of MSG, indicating a concentration-dependent increase in its percentage of inhibition, similar to that exhibited by the standard antioxidant, garlic acid. However, it is noteworthy that monosodium glutamate demonstrated an increase in the scavenging activities as the concentration increased, ranging from 4.61 at 20mg/ml to 35.77 at 100mg/ml.

Table 3: Nitric oxide (NO) scavenging activities of monosodium glutamate and garlic acid

Concentration (ug/ml)	Standard (Garlic Acid)	MSG
20	72.80 ± 7.99	4.61± 2.46*
40	84.14±3.24	12.64± 2.54*
60	86.20 ± 2.48	20.16± 2.75*
80	90.21 ± 0.44	29.83 ± 1.99*
100	91.75 ± 0.07	35.77 ± 4.39*

Values are expressed as mean ± SEM of the different five concentrations.
***Significantly different from the standard at P< 0.05**

Hydroxyl (OH) radical scavenging activities of monosodium glutamate and garlic acid

Table 4 displays the hydroxyl (OH) radical scavenging capacity of monosodium glutamate (MSG). The results demonstrate that the scavenging activity increases as the concentration of MSG increases. However, it is important to note that there is a significant decrease ($p<0.05$) in the scavenging capacity of MSG when compared to the standard antioxidant, garlic acid.

Table 4: Hydroxyl (OH) radical scavenging activity of monosodium glutamate and garlic acid

Concentration (ug/ml)	Standard (Garlic Acid)	MSG
20	50.22±5.04	4.87±2.31*
40	63.92± 8.43	14.38±1.54*
60	72.39 ±5.49	22.13±1.62*
80	80.45 ±5.19	29.13±2.39*
100	84.18 ± 4.30	38.87±2.60*

Values are expressed as mean ± SEM of the different five concentrations.
***Significantly different from the standard at P< 0.05**

Lipid peroxidation (LPO) inhibition activities of monosodium glutamate and garlic acid

Table 5 below presents the antioxidant activity of monosodium glutamate (MSG) in terms of lipid peroxidation inhibition. The results indicate a concentration-dependent increase in the antioxidant activity of MSG. However, it is worth noting that there is a significant decrease in the antioxidant activity of MSG when compared to the standard antioxidant, catechin.

Table 5: LPO inhibition antioxidant activity of monosodium glutamate and garlic acid

Concentration (ug/ml)	Standard (Catechin)	MSG
20	58.91±0.03	11.32±1.42*
40	73.17±3.09	20.81±2.18*
60	78.67±1.86	29.19±2.46*
80	85.30± 2.39	36.69±1.97*
100	87.86±2.18	45.81±1.40*

Values are expressed as mean ± SEM of the different five concentrations.
***Significantly different from the standard at P< 0.05**

In-vivo Antioxidant activities test and Malondialdehyde levels of monosodium glutamate

Table 6 displays the levels of superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and malondialdehyde (MDA) in the rats. The results reveal that in groups B and C, there is an increase of 7.06% and 20.15% in catalase levels respectively, while group D shows a decrease of 13.23% compared to the control group A. However, none of these differences are statistically significant ($p<0.05$).

Furthermore, it was observed that there is a general increase in superoxide dismutase levels in all the groups compared to the control. Group B exhibited the most pronounced increase at 13.72%, while groups C and D showed increases of 4.8% and 4.29% respectively. However, none of these differences were found to be statistically significant in all the groups.

Regarding glutathione levels, group B showed a significant increase of 35.04% ($p<0.05$), whereas groups C and D indicated decreases of 13.7% and 6.5% respectively. It is worth noting that the decrease observed in group D was not statistically significant when compared to the control (group A).

The levels of malondialdehyde demonstrated a decrease of 20.26% in group B, while groups C and D exhibited increases of 12.92% and 25.11% respectively. The increase in group D, which received the highest dose, was particularly notable. All of these results showed significant differences at $p<0.05$.

Table 6: Level of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) enzymes and malondialdehyde (MDA) in the male Sprague-Dawley rats treated with Monosodium glutamate (MSG) compare to the control.

GROUP	GSH (µmol/ml)	SOD (µmol/ml/min/mg pro)	CAT (µmol/ml/min/mg pro)	MDA (µmol/ml)
A	33.35±0.24	2.92± 0.28	17.84±2.63	3.41±0.08
B	45.03±1.24*	3.32± 0.02	19.10±0.32	2.72±0.10
C	28.78± 0.31	3.06±0.05	21.44±0.21	3.85±0.12
D	31.18± 1.01	3.04±0.02	15.48 ± 0.02	4.26±0.05*

Values are expressed as mean ± SEM in each group.
***Significantly different from the standard at P< 0.05**

Discussion

Various physiological activities lead to the production of free radicals, which as an atom or molecule has an unpaired electron and is therefore unstable. These free radicals have tendency to become stable through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells[2] leading to the overproduction of reactive oxygen species (ROS) in plants and animals which causes oxidative stress. This oxidative stress causes damage to tissues and results in large number of diseases. Food additives including monosodium glutamate (MSG) has been linked to oxidative stress[3]. In general, there has been extensive study on the use of monosodium glutamate as a seasoning and flavor enhancer. These studies have dwell on

the possible adverse health effects that the salt might pose to the teaming populace as a result of changes in the lifestyle. Consumption of MSG in food has been linked to induction of oxidative stress in animals[24, 25.]

This study, however, investigated the potential antioxidant properties of monosodium glutamate by assessing its *in-vitro* and *in-vivo* antioxidant activities in rats. Results from the *in-vitro* study reveal high DPPH scavenging activity at a concentration of 100µg/ml. It means that MSG exhibited free radical scavenging activity but at lower strength when compared to the standard, garlic acid. Similar results were observed with the ferric reducing antioxidant power (FRAP), nitric oxide (NO) scavenging activity, hydroxyl (OH) radical scavenging activity, and lipid peroxidation (LPO) inhibition activity. The reducing capacity of this compound could serve as an indicator of potential antioxidant property.

Furthermore, the results from the *in-vivo* antioxidant activities showed an increase in serum malondialdehyde (MDA) concentration, a marker of lipid peroxidation (LPO), in MSG-treated rats (groups C and D) in the present study, can be said to be as a result of generation of reactive oxygen species (ROS) as previously suggested by Droge[24.] High glutamate metabolism leads to calcium ion influx (Ca^{2+}) in mitochondria which will eventually cause increase in the ROS production[26.] The observed increase in the serum marker of LPO by MSG appears to confirm an earlier report by Rajagopal *et al*., [25] that the administration of MSG induced oxidative stress in experimental animals. However, the result for group B showed that there is a significant reduction ($p<0.05$) in the MDA level, which could be as a result of it being the group that received the lowest dose (500mg/Kg).

Superoxide dismutase (SOD) is the first line of antioxidant defense mechanism that neutralizes the oxidant rapidly[27.] Therefore, the rise in SOD activity as the dosage of MSG increases in this study might be due to the compensatory mechanism as the enzymatic antioxidant was regulating the increased production of ROS[26.] However, this result is not in conformity with other previous studies that showed a decreasing activity of SOD in rats[28.] The observed difference could be attributed to the dose and duration of treatment.

The catalase (CAT) activity is necessary for converting hydrogen peroxide into water. The CAT in the current study increased in MSG induced groups which may be suggestive of compensatory mechanism towards alleviating the effect of oxidative stress. The increased level of CAT in the MSG treated animals is an indication of efficiency of CAT activity in the degradation of hydrogen peroxide. Toxic hydroxyl radicals which contribute significantly to oxidative stress can be generated from hydrogen peroxide[26.]

The glutathione (GSH) level show significant changes ($p < 0.05$) among all the treatment groups as shown in table 4.7. The results for groups C and D show a decrease which is significant only in group C when compared to the control. This is in agreement with other studies that also showed decreased in GSH level of MSG-treated rats [24]. The decrease in GSH level in this study could be due to the alteration of glutamate cysteine anti porter system at the cell membrane. This system provides medium of exchange between intracellular glutamate and the extracellular cysteine which involves in the synthesis of GSH[26, 29.]

Conclusion

Results from this study revealed that MSG possesses free radical scavenging activities as well as lipid peroxidation inhibition property, but at a lower strength relative to their respective standards. Therefore, MSG can be said to have some potential antioxidant property. Also, as demonstrated by the increasing levels of antioxidants enzymes (SOD, CAT) it can be said that it is safe to consume monosodium glutamate (MSG) at a dose not exceeding 24% LD_{50} as used in this study. MSG is, therefore, safe at a limited usage as increased consumption can lead to potential health hazards as a result of induced oxidative stress.

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