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Ameliorative Effects of Calcium-Based Supplements on Monosodium Glutamate-Induced Organ Injury in Rats

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ABSTRACT

Monosodium glutamate (MSG), is the sodium salt of the amino acid; glutamic acid. MSG is known to improve food flavours, yet it poses significant health risks to consumers. This study aims to explore the potential ameliorative effect of calcium-based supplements against MSG-induced organ damage in rats. The experiment was divided into two phases; Phase I (Pre-MSG induction), and Phase II (Post-MSG induction). Ninety-six (96) female Wistar rats weighing between 100 and 120 g were allotted into five groups of nine rats each in each of the two phases. A daily dose of 750 mg/kg of MSG was administered intraperitoneally to the rats to induce tissue injury. Calcium-based supplements, including calcium, calcium D-glucarate, and the combination of both were administered to the rats prior to MSG exposure (Phase I), and after MSG exposure (Phase II). After 14, 28, and 42 days of treatment, the rats in both phases were sacrificed, and selected organs (brain, liver, and uterus) were harvested, and used for biochemical analysis, focusing on antioxidants biomarkers including catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels. Results indicated that the pre- and post-administration of calcium-based supplements significantly mitigated MSG-induced tissue damage by increasing catalase, and glutathione peroxidase activities, and decreased MDA levels, particularly in the brain and liver both pre- and post-administration, suggesting a potential protective effect against MSG-induced organ injury by enhancing endogenous antioxidant activity. In conclusion, the findings from this study suggest that calcium-based supplements may offer protection against MSG-induced organ damage by modulating antioxidant mechanisms.

Keywords: Calcium D-glucarate, Monosodium glutamate, Calcium, Antioxidant levels, Calcium-based supplements.

Introduction

Monosodium glutamate (MSG) is the sodium salt of L-glutamic acid, which occurs naturally in many foods.[1] MSG imparts a unique taste that cannot be replicated by other meals. It produces a distinct flavour known in Japanese as umami, which means "savoury".[2] MSG is popularly used in the food industry, as well as in household kitchens, and restaurants. Flavoured chips and cookies, dried or packaged sauces or soups, fresh sausages, marinated meats, premade foods, frozen foods, stuffed chicken, oriental sauce or bottled soy sauce, processed meats, some hams, flavoured sushi, and organic burgers all contain this flavour enhancer.[3] MSG is used as a pure salt additive in food or in amino acid mixtures.[4] MSG intake has risen worldwide in recent years as a flavouring to improve palatability and meal selection. Several studies have investigated the toxic and metabolic properties of MSG, with numerous findings indicating that repeated levels of MSG can cause oxidative stress in various organs and tissues of laboratory animals.[5]

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The most commonly reported effects of MSG use include toxic effects to the nervous system, toxic effects on genital organs, metabolic abnormalities, obesity, and oxidative stress.[6,7]
Consumer demand for packaged food items has risen dramatically due to rapid population growth and evolving lifestyles. The widespread use of food additives, and excessive consumption of packaged and processed foods has led to negative health impacts. Mutagenicity, hypersensitivity, genotoxicity, and obesity are just some of the potential adverse effects of frequent consumption of food additives and processed foods. Due to their harmful effects, certain food additives have been banned.[8]
Beyond its well-established functions in osteoporosis prevention and therapy, calcium has non-skeletal effects, particularly cardiovascular effects, which is increasingly controversial. Calcium supplementation has been related to a reduced risk of heart disease in several studies.[9]
The process by which calcium impacts its cardiovascular effect appears to be through changes in glucose metabolism and lipid profiles, as well as reduction of blood pressure.[10]
Calcium D-Glucarate is a natural chemical produced in small amounts by the human body and is also contained in vegetables and fruits, including apples, oranges, grapefruit, broccolis, cod, and kale. Calcium Glucarate or D-glucarate is commonly referred to as CDG. Calcium glucarate, a D-glucaric acid derivative, helps support the body's defence against toxins and excess steroid hormones, making it useful for supporting the detoxification role of the liver and potentially protecting against suboptimal health caused by hormone imbalances and toxic burden. Glucuronidation - a process in which a chemical called glucuronic acid is bonded to a toxin in order to form a molecule that is more easily excreted by the body – is used to clear some toxins during regular liver detoxification.[11] The objective of this study was to confirm the deleterious impact of MSG on various organs and the ameliorative

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effect of calcium D-glucarate and calcium on MSG-induced toxicity, by measuring the levels of antioxidant markers.

Materials and Methods

Animals and experimental protocol

Ninety-six (96) female rats, each weighing between 100 - 120 g were obtained from the University of Agriculture, Abeokuta, Ogun State, Nigeria. The rats were kept in cages at room temperature in the animal house, and were left to acclimatize to the laboratory condition for two (2) weeks. They were fed with normal rodent pellets, and allowed free access to drinking water.

Ethical approval

Ethical approval was obtained from the Covenant Health Research and Ethics Committee (CHREC), Canaanland prior to the commencement of the experiment. Ethical permit with reference number CHREC/066/2021 was granted.

Induction of toxicity using monosodium glutamate

A dose of 750 mg/kg of monosodium glutamate solution was administered to the rats via the intraperitoneal route once daily for 42 days to induce tissue toxicity. At two weekly intervals; on day 14, day 28, and day 42, the animals were subjected to 24-hour fasting after which they were sacrificed under xylazine and ketamine anaesthesia.

Experimental design and administration of calcium-based supplements

The experiment was divided into two phases; the Pre-MSG induction phase (Phase I), and the Post-MSG induction phase (Phase II). The rats were allotted into five groups of nine animals each in each of the two phases of the experiment.

Calcium D-glucarate (440 mg/L in distilled water) were prepared such that 9.2 µL/g bwt of rats is administered orally once daily using an oro-gastric tube. The protocol for the administration were as follows:

Phase I: Pre-MSG induction phase

Group 1 - administered distilled water to serve as normal control.

Group 2 - administered 750 mg/kg bwt monosodium glutamate to serve as negative control.

Group 3 - administered calcium D-glucarate (35.0 mg/kg bwt.) formulated diet and subsequently the daily administration of 750 mg/kg bwt monosodium glutamate.

Group 4 - administered calcium (9.2 µL/g bwt.) formulated diet and subsequently the 750 mg/kg bwt. monosodium glutamate.

Group 5 - administered a combination of the calcium formulated diet (9.2 µL/g bwt.) and the calcium D-glucarate supplement (35.0 mg/ kg bw), followed by 750 mg/kg bwt. monosodium glutamate.

Phase II: Post-MSG induction phase

Group 1 - administered distilled water to serve as normal control.

Group 2 - administered 750 mg/kg bwt monosodium glutamate to serve as negative control.

8. Group 3 - administered 750 mg/kg bwt monosodium glutamate and subsequently the calcium D-glucarate-based diet (35.0 mg/kg bw).

Group 4 - administered 750 mg/kg bwt monosodium glutamate, and subsequently the Calcium-based diet (9.2 µL/g bwt.).

Group 5 - administered 750 mg/kg bwt monosodium glutamate, and subsequently combination of the calcium formulated diet (9.2 µL/g bwt.) and the calcium D-glucarate supplement (35.0 mg/ kg bw).

Organ preparation for analysis

The uterus, liver and brain were harvested following the method outlined by Afolabi *et al*., (2018).[12] At the end of each treatment phase; on the 14th day, 28th day, and 42nd day, the organs were removed, washed with normal saline and stored at −20°C. Prior to analysis, the organs were weighed, then kept in buffer solution. A section of the organ (0.2 g) was homogenized in 500 uL of lysis buffer (50 mM potassium phosphate buffer, pH 7.4). The homogenate was centrifuged at 4000 x g for 15 min at 4ºC min, the supernatant was collected and preserved at -80ºC until used for analysis.[12]

Determination of antioxidant activity

The levels of antioxidant enzymes [Catalase, Glutathione peroxidase, and Malondialdehyde (MDA)] were measured using enzyme linked immunosorbent assay (ELISA), following manufacturer's instructions. Absorbance readings were taken using a spectrophotometer (Microplate Reader ELx 800TM BioTek®, Seattle, WA, USA).

1. Determination of catalase activity

The catalase activity was measured using the technique published by Bock *et al*. (1980).[13] To 100 uL of homogenate and 400 uL of distilled water, 500 uL of phosphate buffer was added. The mixture was incubated at 37°C for 2 min. The mixture (1 mL) was added to 500 uL of hydrogen peroxide in a cuvette, and the absorbance was measured at 240 nm at zero time (AI) and after 30 minutes (A2) against a blank (distilled water). The catalase activity (µmol/sec/g) was calculated according to the formula;

Catalase Activity = K/T x 10/g fresh tissue x log A1/A2

Where;
K = Constant = 2.3,
T = Time in seconds,
A1 = Sample absorbance at zero time,
A2 = Sample absorbance after 30 minutes.

2. *Determination of glutathione peroxidase (GPx) activity*

The assay measures the activity of cellular (c-GPx) in an indirect manner. The enzyme glutathione reductase recycles oxidised glutathione (GSSG) generated by reducing an organic peroxide to its reduced state (GR) by c-GPx.[14] The GPx activity was measured using the technique given by Chance and Maehly (1955).[15] Briefly, 1.5 mL of freshly prepared peroxidase buffer (50 mL of 0.4 mM phosphate buffer + 315 mg pyrogallol, pH 7) and 0.25 mL of 1% H_2O_2 solutions were added in tubes. To start the reaction, 50 µL of the test sample was added to the solution. Increase in absorbance readings were recorded (against blank containing distilled water) at different intervals for 5 min at 420 nm. The GPx activity was calculated using the following equation;

$$\text{GPx activity (Units/mL)} = \frac{(\Delta A\ 420\ \text{nm/min Test} - \Delta A\ 420\text{nm/min blank})\ (1.8)(\text{df})}{0.05 \times 12}$$

Where;
df = Dilution factor,
12 = Millimolar extinction coefficient of 1 mg/mL of purpurogallin at 420 nm,
1.8 = Total vol (mL) of assay,
0.05 = Volume (mL) of enzyme used.

3. Determination of lipid peroxidation product (MDA)

In the catabolism of lipid peroxides, MDA and thiobarbituric acid (TBA) react to produce a red molecule with a maximum absorption at 532 nm.

The malondialdehyde (MDA) concentration (product of lipid peroxidation) in the liver was ascertained as thiobarbituric acid reactive substance (TBARs) based on the modification of the method of Ohkawa *et al*. (1979).[16] Briefly, a stock solution was prepared by dissolving 0.375% (w/v) thiobarbituric acid, 15% (w/v) trichloroacetic acid, and 0.25 M hydrochloric acid in 50 mL of distilled water. Supernatant of the tissue homogenate (0.5 mL) was thoroughly mixed with 1 mL of stock solution and then heated in a boiling water bath for 30 minutes. After cooling, the clear supernatant was extracted, and centrifuged at 2000 r.p.m for 15 minutes.

The MDA concentration was calculated using the following equation;

$$\text{MDA concentration (nmol MDA/g tissue)} = \frac{A_{532}}{\varepsilon \times L}\ x\ \frac{V_{reaction}}{Tissue\ weight}$$

Where;
A_{532} = Absorbance at 532 nm,
ε = The extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$,
L = Path length of the cuvette,
$V_{reaction}$ = Total volume of the reaction mixture,
Tissue weight = Weight of the tissue sample used for the assay.

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Statistical analysis
The experiments were performed in triplicate, and the results were reported as mean ± standard deviation. Statistical analysis was done using Megastat software. Data were subjected to one-way analysis of variance (ANOVA), and post hoc pairwise T-test for multiple comparison. $P < 0.05$ was considered statistically significant.

Results and Discussion

MSG is a flavour enhancer, and a food additive that is widely used in various food industries. Recently, the toxicity and safety of MSG is now the focus of many studies. Nowadays, modern nutrition involves continuous intake of this flavour enhancers, suggesting cumulative increase of glutamic acid (GA) level in the blood.[8] This study elucidates the protective role of calcium (Ca^{2+}), and calcium D-glucarate against MSG-induced organ damage by measuring the status of oxidants/antioxidant enzymes before and after administration of calcium, calcium D-glucarate, and a combination of both.

Effect of calcium and calcium D-glucarate on catalase (CAT) activity
CAT reduces hydrogen peroxide (H_2O_2) build up in the cell by dismutating it to generate water (H_2O) and oxygen (O_2), or by acting as an antioxidant, wherein it functions as a peroxidase.[17] The pre-administration of the combination of calcium and calcium D-glucarate supplements significantly ($p < 0.05$) reduced CAT activity in the brain tissue on day 14 following MSG induction compared to the corresponding control (Table 1). This initial decrease in catalase enzyme activity could be due to excessive H_2O_2 levels in the brain tissue, which then result in the generation of hydroxyl radicals (·OH), which then causes the initiation and propagation of lipid peroxidation.[17] The brain comprises high amounts of poly-unsaturated fatty acids (PUFA), that are particularly susceptible to attacks by free radicals leading to the formation of hydroxyl and alkyl radicals.[17] This initial decrease in catalase activity was restored to normal levels by the pre-administration of the combined calcium and calcium D-glucarate supplements for the remaining days of the experiment.
The liver plays a vital role in metabolism, and has several other functions, including plasma protein synthesis, glycogen storage, bile formation, and most importantly chemical detoxification. The liver is vulnerable to damage from toxic chemicals since it is involved in so many activities.[18]
In this study, there was a significant ($p < 0.05$) reduction in catalase activity in the liver at day 28 following MSG treatment. However, the pre-administration of calcium alone and the combination of calcium and calcium D-glucarate resulted in a significant increase in catalase activity in the liver at the end of the 42 days treatment period (Table 1).
The increase in antioxidant enzymes activities might be a response to oxidant treatment and the adaptation to cope with oxidative stress.[19] These supplements (calcium alone and the combination of calcium and calcium D-glucarate) may therefore possess potential protective effect on the liver. For the uterine tissue, neither the pre-administration of calcium and calcium D-glucarate supplements nor MSG treatment was capable of eliciting changes in catalase activity in the uterus throughout the 42-day study period (Table 2). It is important to note that, in the three organs (brain, liver, and uterus), the post-treatment with calcium or combination of calcium and calcium D-glucarate resulted in no significant changes in catalase activity throughout the 42-day experimental period (Table 2).

Effect of calcium and calcium D-glucarate on glutathione peroxidase (GPx) activity
GPx catalyses the reduction of peroxide molecules (ROOH and H_2O_2) making use of glutathione as the substrate. It therefore protects mammalian cells from oxidative stress. Study has shown that a decrease in GPx activity could make the tissue more susceptible to damage from lipid peroxidation.[20]
In the present study, the pre-administration of calcium alone, calcium D-glucarate alone, the combination of calcium and calcium D-glucarate as well as the negative control (MSG Group) significantly ($p < 0.05$) reduced GPx activity in the brain for the first 14 days period.

Table 1: Effects of pre-administration of calcium supplements on catalase activity in some organs of monosodium glutamate treated rats

Parameter	Specific Activity (µmol/mg protein) x 10^{-2}		
	Brain	**Uterus**	**Liver**
C-14-PrT	3.25 ± 2.11	4.57 ± 1.26	2.21 ± 0.89
MG-14-PrT	2.16 ± 1.50	1.09 ± 0.58	4.20 ± 2.75
MG-14-Ca-PrT	1.68 ± 1.36	8.60 ± 1.87	3.15 ± 0.23
MG-14-DG-PrT	3.67 ± 1.36	3.94 ± 1.00	3.65 ± 1.85
MG/Ca 14 DG PrT	1.11 ± 0.49 [a]	4.73 ± 2.99	4.36 ± 3.57
C-28-PrT	0.74 ± 0.44	5.40 ± 2.20	9.20 ± 2.55
MG-28-PrT	2.70 ± 0.09	3.89 ± 1.95	1.57 ± 1.35 [a]
MG-28-Ca-PrT	2.33 ± 1.55	7.50 ± 0.04	2.15 ± 1.58 [a]
MG-28-DG-PrT	2.00 ± 0.00	15.82 ± 0.00	6.08 ± 0.00
MG/Ca 28 DG PrT	2.96 ± 1.28	2.10 ± 1.23	3.82 ± 1.32 [a]
C-42-PrT	2.27 ± 1.71	15.54 ± 6.20	2.51 ± 1.11
MG-42-PrT	3.90 ± 0.59	4.70 ± 1.10	1.67 ± 1.41
MG-42-Ca-PrT	1.33 ± 12.79	11.17 ± 6.70	8.07 ± 2.75 [a]
MG-42-DG-PrT	4.17 ± 0.82	11.10 ± 8.15	2.12 ± 0.44
MG/Ca 42 DG PrT	1.64 ± 0.67	22.49 ± 10.95	7.30 ± 2.25 [a]

Values are mean ± standard deviation (SD). C = Normal control, MG = Monosodium glutamate, Ca = Calcium. DG = Calcium D-Glucarate, PrT = Pre-treatment. The numbers 14, 28, 42 within the parameters represent day 14, day 28, and day 42, respectively.

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Table 2: Effects of post-administration of calcium supplements on catalase activity in some organs of monosodium glutamate treated rat

Parameter	Specific Activity (µmol/mg protein) x 10^{-2}		
	Brain	**Uterus**	**Liver**
C-14-PstT	1.13 ± 0.46	6.35± 1.65	20.94 ± 9.82
MG-14-PstT	9.34 ± 5.14	3.93 ± 3.79	2.78 ± 0.69
MG-14-Ca-PstT	12.79 ± 7.04	11.20 ± 6.52	9.59 ± 4.03
MG-14-DG-PstT	1.48 ± 0.73	11.06 ± 5.85	12.51 ± 5.90
MG/Ca 14 DG PstT	7.56 ± 3.74	4.68 ± 1.72	8.19 ± 3.61
C-28-PstT	1.67 ± 0.61	7.82 ± 1.29	2.51 ± 0.67
MG-28-PstT	2.59 ± 1.11	6.06 ± 3.88	5.31 ± 2.32
MG-28-Ca-PstT	3.88 ± 1.73	0.46 ± 0.23	4.57 ± 1.59
MG-28-DG-PstT	4.40 ± 1.57	10.80 ± 3.33	5.78 ± 1.28
MG/Ca 28 DG PstT	3.58 ± 0.76	9.38 ± 2.19	3.96 ± 1.79
C-42-PstT	0.95 ± 0.24	4.99 ± 2.01	3.52 ± 0.87
MG-42-PstT	0.89 ± 0.82	1.18 ± 0.20	1.70 ± 0.76
MG-42-Ca-PstT	5.99 ± 2.75	1.21 ± 0.40	3.96 ± 0.74
MG-42-DG-PstT	1.63 ± 0.73	5.86 ± 1.96	4.76 ± 1.83
MG/Ca 42 DG PstT	5.63 ± 3.01	7.46 ± 4.31	3.59 ± 0.98

Values are mean ± standard deviation (SD). C = Normal control, MG = Monosodium glutamate, Ca = Calcium. DG = Calcium D-Glutarate, PstT = Post-treatment. The numbers 14, 28, 42 within the parameters represent day 14, day 28, and day 42, respectively.

Table 3: Effects of pre-administration of calcium supplements on peroxidase activity in some organs of monosodium glutamate treated rats

Parameter	Specific Activity (U/mg protein) x 10^{-2}		
	Brain	**Uterus**	**Liver**
C-14-PrT	3.01 ± 2.75	3.17 ± 1.49	8.60 ± 0.85
MG-14-PrT	0.79 ± 0.10[a]	0.78 ± 0.51	4.47 ± 0.53
MG-14-Ca-PrT	0.61 ± 0.26 [a]	0.86 ± 0.33	4.45 ± 1.43
MG-14-DG-PrT	0.55 ± 0.02[a]	1.04 ± 0.26	5.89 ± 2.34
MG/Ca 14 DG PrT	0.59 ± 0.06[a]	0.76 ± 0.09	5.81 ± 4.22
C-28-PrT	0.54 ± 0.13	3.38 ± 1.59	3.49 ± 0.72
MG-28-PrT	1.32 ± 0.428	2.77 ± 1.20	4.30 ± 1.98
MG-28-Ca-PrT	0.48 ± 0.05	9.522 ± 4.41	8.67 ± 0.58
MG-28-DG-PrT	0.55 ± 0.00	1.11 ± 0.00	16.50 ± 0.00 [a]
MG/Ca 28 DG PrT	0.53 ± 0.00	0.68 ± 0.30	6.19 ± 2.24
C-42-PrT	0.94 ± 0.53	5.97 ± 2.70	7.88 ± 3.41
MG-42-PrT	1.65 ± 0.17	0.79 ± 0.20	4.08 ± 1.37
MG-42-Ca-PrT	1.95 ± 0.71 [a]	3.43 ± 1.82	8.88 ± 1.12
MG-42-DG-PrT	0.54 ± 0.098	0.95 ± 0.09	59.89 ± 9.63[a]
MG/Ca 42 DG PrT	0.65 ± 0.16	0.92 ± 0.29	5.50 ± 2.11

Values are mean ± standard deviation (SD). C = Normal control, MG = Monosodium glutamate, Ca = Calcium. DG = Calcium D-Glucarate, PrT = Pre-treatment. The numbers 14, 28, 42 within the parameters represent day 14, day 28, and day 42, respectively.

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Whereas, at the end of the 42 days study period, the pre-administration of calcium alone significantly increased GPx activity compared to the corresponding control in the brain (Table 3). Low GPx activity could make the tissue more vulnerable to lipid peroxidative damage beyond the 42 days period of the study.[20] However, the post-administration of calcium alone, the combination of calcium and calcium D-glucarate supplements, as well as MSG treatment only, all significantly increased GPx activity in the brain during the first 14 days compared to the control (Table 4).

For the liver sample, the pre-administration of calcium D-glucarate alone increased GPx activity between 28 – 42 days administration (Table 3). On the other hand, post-administration of calcium alone, and the combination of calcium and calcium D-glucarate significantly increased GPx activity in the liver at the initial 14 days period. Similarly, MSG treatment alone elicited an increase in GPx activity between the 14-28 days (Table 4). The overall increase in GPx activity may be a reaction to antioxidant treatment or an adaptation meant to help the body deal with oxidative stress. Furthermore, it can be due to the enhanced synthesis of enzymes through induction.[21] However, at the end of the 42-day treatment, it was observed that the post-administration of all the supplements (calcium alone, calcium D-glucarate alone, and the combination of calcium and calcium D-glucarate), as well as the negative control (MSG treatment Group) significantly ($p < 0.05$) decreased GPx activity in the liver (Table 4).

In the uterus, the pre-administration of all the three calcium-based supplements elicited no GPx response throughout the 42-day study period (Table 3), while the post-administration of calcium alone significantly reduced GPx activity, and calcium D-glucarate alone increased the GPx activity in the uterus between the 28-42 days treatment period (Table 4). The observed low GPx activity during the post administration of the calcium-based supplements alone was not sufficient to provoke a high lipid peroxidation level within the 42 days period of experiment. However, it may render the tissue more susceptible to lipid peroxidation damage in the liver following an extended treatment beyond the 42-day period of the study.[22]

Table 4: Effects of post-administration of calcium supplements on peroxidase activity in some organs of monosodium glutamate treated rats

Parameter	Specific activity (U/mg protein) x 10^{-2}		
	Brain	**Uterus**	**Liver**
C-14-PstT	0.68 ± 0.22	1.90 ± 0.33	1.78s ± 0.57
MG-14-PstT	3.24 ± 0.10[a]	2.13 ± 0.80	2.14 ± 0.81
MG-14-Ca-PstT	2.32 ± 0.20[a]	2.24 ± 1.22	3.07 ± 0.76
MG-14-DG-PstT	0.50 ± 0.18	1.32 ± 0.28	0.66 ± 0.13
MG/Ca 14 DG PstT	3.00 ± 1.90[a]	2.00 ± 0.75	0.63 ± 0.03
C-28-PstT	0.36 ± 0.13	1.52 ± 0.17	2.74 ± 0.99
MG-28-PstT	1.67 ± 0.33[a]	0.85 ± 0.32	0.66 ± 0.19
MG-28-Ca-PstT	0.50 ± 0.03	0.05 ± 0.02 [a]	2.19 ± 0.76
MG-28-DG-PstT	0.52 ± 0.07	6.55 ± 0.92 [a]	4.05 ± 4.09
MG/Ca 28 DG PstT	0.58 ± 0.15	0.65 ± 0.04	0.55 ± 0.15
C-42-PstT	0.53 ± 0.01	2.83 ± 0.09	3.34 ± 0.67
MG-42-PstT	0.72 ± 0.15	3.48 ± 1.59	0.63 ± 0.25 [a]
MG-42-Ca-PstT	0.66 ± 0.16	1.56 ± 0.40	0.70 ±.0.09 [a]
MG-42-DG-PstT	0.16 ± 0.21	0.98 ± 0.31 [a]	0.93 ± 0.23 [a]
MG/Ca 42 DG PstT	0.71 ± 0.03	3.41 ± 0.32	0.65 ± 0.15 [a]

Values are mean ± standard deviation (SD). C = Normal control, MG = Monosodium glutamate, Ca = Calcium. DG = Calcium D-Glutarate, PstT = Post-treatment. The numbers 14, 28, 42 within the parameters represent day 14, day 28, and day 42, respectively.

Effect of calcium and calcium D-glucarate on malondialdehyde (MDA) levels

Oxygen radicals may cause lipid peroxidation (LPO) of biomembranes via chain reactions. The first step is the initiation reaction, which involves the removal of hydrogen atoms from polyunsaturated fatty acids (PUFA) by an oxygen radical. The second phase is propagation, and the last phase is termination. The level of LPO is mostly determined by the level of thiobarbituric acid (TBA), which is estimated using malondialdehyde.[22]

Calcium alone, and calcium D-glucarate alone, as well as the negative control (MSG) significantly increased the MDA level in the initial 14 days pre-treatment phase. This may induce the bio-membrane susceptibility, and eventually tissue damage/injury.[17] On the contrary, the pre-administration of calcium alone significantly reduced liver MDA levels only at day 42. This suggest that the initially induced peroxidation was possibly rectified beyond the 14 days pre-administration of calcium alone as depicted by the reduced MDA levels in the liver following the 42 days period (Table 5). Similarly, the MDA levels in the liver were reduced by the post administration of calcium alone throughout the 42 days of the study. Whereas, the calcium D-glucarate alone was only able to reduce MDA levels for the initial 14 days, and their combination (calcium and calcium D-glucarate) was able to reduce MDA levels beyond the initial 28 days (i.e. 28-42 days). The MSG was also able to reduce the MDA levels in the liver only during the initial 14 days post administration (Table 6).

The 42 days pre-administration of calcium D-glucarate alone and the combination of calcium and calcium D-glucarate significantly reduced the MDA levels in the brain at day 42 (Table 5). All the calcium-based supplements caused no significant alteration in the MDA levels in the brain following their post-administration throughout the 42 days study period (Table 6).

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In the uterine tissue, there were no significant differences in the MDA levels across all the calcium-based supplements groups following their pre-administration all through the study period (Table 5). MSG treatment only significantly increased the MDA levels in the uterus at the initial 14 days of treatment, but the post-administration of all the calcium-based supplements (calcium alone, calcium D-glucarate alone, and the combination of calcium and calcium D-glucarate) significantly reduced the MDA level in the uterus from the 28th to the 42nd day of the study (Table 6).

Table 5: Effects of pre-administration of calcium supplements on malondialdehyde levels in some organs of monosodium glutamate treated rats

Parameter	Malondialdehyde Levels (µmol/mg protein)		
	Brain	Uterus	Liver
C-14-PrT	42.55 ± 14.21	209.47 ± 46.98	42.79 ± 2.04
MG-14-PrT	58.91 ± 10.69	145.99 ± 50.00	110.45 ± 42.79 [a]
MG-14-Ca-PrT	48.85 ± 13.82	71.54 ± 42.42	116.57 ± 48.87 [a]
MG-14-DG-PrT	41.63 ± 19.19	72.15 ± 31.17	113.43 ± 64.49 [a]
MG/Ca 14 DG PrT	59.74 ± 26.65	44.68 ± 5.32	33.01 ± 8.80
C-28-PrT	41.54 ± 6.53	134.28 ± 62.21	79.52 ± 6.66
MG-28-PrT	54.84± 23.12	162.79 ± 51.22	82.69 ± 10.65
MG-28-Ca-PrT	32.93 ± 8.36	55.96 ± 7.89	41.20 ± 2.52
MG-28-DG-PrT	40.77 ± 1.09	63.27 ± 0.00	27.26 ± 0.74
MG/Ca 28 DG PrT	51.49 ± 6.46	70.67 ± 27.99	37.98 ± 12.51
C-42-PrT	51.49 ± 9.99	134.95 ± 23.05	101.79 ± 44.53
MG-42-PrT	56.25 ± 3.94	168.56 ± 81.05	128.80 ± 18.29
MG-42-Ca-PrT	25.74 ± 2.46	83.14 ± 66.74	31.28 ± 3.67 [a]
MG-42-DG-PrT	24.90 ± 3.08 [a]	80.45 ± 31.13	44.55 ± 15.56
MG/Ca 42 DG PrT	22.47 ± 2.50 [a]	41.41 ± 15.98	44.17 ± 7.04

Values are mean ± standard deviation (SD). C = Normal control, MG = Monosodium glutamate, Ca = Calcium. DG = Calcium D-Glucarate, PrT = Pre-treatment. The numbers 14, 28, 42 within the parameters represent day 14, day 28, and day 42, respectively.

Table 6: Effects of post-administration of calcium supplements on malondialdehyde levels in some organs of monosodium glutamate treated rats

Parameter	Malondialdehyde Levels (µmol/mg protein)		
	Brain	Uterus	Liver
C-14-PstT	29.66 ± 9.18	83.56 ± 26.52	141.73 ± 44.74
MG-14-PstT	35.13 ± 4.31	120.35 ± 28.01[a]	75.90 ± 6.31[a]
MG-14-Ca-PstT	22.21 ± 7.85	114.90 ± 4.56	63.69 ± 26.48 [a]
MG-14-DG-PstT	32.88 ± 16.65	99.71 ± 13.32	136.63 ± 20.59 [a]
MG/Ca 14 DG PstT	68.08 ± 51.92	98.43 ± 10.12	74.58 ± 6.11
C-28-PstT	59.42 ± 14.14	119.47 ± 7.28	117.64 ± 33.66
MG-28-PstT	38.43 ± 9.42	125.16 ± 8.18	92.28 ± 18.25
MG-28-Ca-PstT	36.79 ± 4.17	43.53 ± 16.49 [a]	38.85 ± 9.49[a]
MG-28-DG-PstT	49.07 ± 5.52	41.83 ± 7.26 [a]	66.67 ± 34.15
MG/Ca 28 DG PstT	36.09 ± 0.73	41.83 ± 19.33 [a]	59.97 ± 53.99[a]
C-42-PstT	40.38 ± 6.94	155.58 ± 18.09	108.61 ± 17.34
MG-42-PstT	39.62 ± 8.02	136.97 ± 8.63	98.17 ± 23.66
MG-42-Ca-PstT	41.35 ± 35.31	72.63 ± 42.61 [a]	61.73 ± 5.77 [a]
MG-42-DG-PstT	65.48 ± 12.66	79.04 ± 11.31 [a]	101.03 ± 33.77
MG/Ca 42 DG PstT	66.73 ± 19.45	67.92 ± 6.03 [a]	95.35 ± 33.46 [a]

Values are mean ± standard deviation (SD). C = Normal control, MG = Monosodium glutamate, Ca = Calcium. DG = Calcium D-Glucarate, PstT = Post-treatment. The numbers 14, 28, 42 within the parameters represent day 14, day 28, and day 42, respectively.

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Conclusion

The findings from the study revealed that calcium-based supplements could possibly prevent organ damage due to MSG consumption, and may also ameliorate or reverse MSG-induced organ damage by their ability to boost antioxidant enzymes activities, and inhibit lipid peroxidation, which was manifested by the reduction of malondialdehyde levels following the pre- and post-administration of these supplements.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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