

## Determination of Nephrotoxicity and Hepatotoxicity of Monosodium Glutamate (MSG) Consumption

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**Abstract:** Investigations were conducted to determine the Nephrotoxicity and Hepatotoxicity of Monosodium Glutamate (MSG). Graded doses of monosodium glutamate were administered orally to Adult Wister rats weighing between (160.7-238.6 g) for 28 days. Liver and Kidney function tests were carried out on the blood serum. Monosodium glutamate elicited an increase in body weight in an ascending fashion from group B to group D though not statistically significant ( $p>0.05$ ) Biochemical parameters (Electrolytes, Urea, Creatinine, RBC, Cholesterol and Enzymes) were not seriously affected though there were increases in the concentration or levels of some of them. This shows that monosodium glutamate has hepatotoxicity and nephrotoxicity tendencies especially when consumed at higher concentrations.

**Key words:** Adult wister rats, hepatotoxicity, monosodium glutamate, nephrotoxicity, serum biochemistry carcinogenic

### INTRODUCTION

Toxicity is defined as any harmful effect of a chemical or a drug on a target organism (Wallace, 1982). Nephrotoxicity (from the Greek; Nephros, "Kidney") is a poisonous effect of some substances, both toxic chemicals and medication on the kidneys ([en.wikipedia.org/wiki/Nephrotoxicity](http://en.wikipedia.org/wiki/Nephrotoxicity)) while Hepatotoxicity (from Hepatic toxicity) implies chemical-driven liver damage ([en.wikipedia.org/wiki/hepatotoxicity](http://en.wikipedia.org/wiki/hepatotoxicity)).

Since the Kidney is involved in the excretion of many toxic metabolic waste products, particularly the nitrogenous compounds and the liver is also involved in many metabolic processes.

Monosodium glutamate otherwise known as Aji-nomoto is the sodium salt of glutamic acids. Glutamate is one of the most common amino acids in nature and is the main component of many proteins and peptides of most tissues. Monosodium glutamate contains 78% glutamic acid, 22% sodium and water (Adrienne, 1999). Glutamate is also produced in the body and plays an important/essential role in human metabolism. It is a major component of many protein-rich food products such as meat, fish, milk and some vegetables (IFIC, 1994). Various environmental chemicals, industrial pollutants and food additives have been implicated as causing harmful effects, (Kirk-Othmer, 1992). Most food additives act either as preservatives, or enhancer of palatability. One such food additive is monosodium glutamate (MSG) and in Nigeria, MSG is sold in the open market stalls and stores as "Ajinomoto marketed by West

African Seasoning Company Limited as "Vedan" or "White Maggi". When monosodium glutamate is added to food, it provides a flavouring function similar to the naturally occurring free glutamate: which differ from the four classic tastes of sweet, sour, salt and bitter (Leung and Foster, 1996). As a food additive, monosodium glutamate is described and listed on food labels as a "Flavouring" or "Hydrolysed vegetable protein". Through its stimulation of the Orosensory receptors and improving the palatability of meals, monosodium glutamate influences the appetite positively and induces weight gain, (Moore, 1999). Despite its taste stimulation, and improved appetite enhancement, reports indicate that monosodium glutamate is toxic to humans and experimental animals, (Biodun and Biodun, 1993).

In Nigeria, most communities and individuals often use monosodium glutamate as a bleaching agent for the removal of stains from clothes. There is a growing apprehension that its excellent bleaching properties could be harmful or injurious to the tissues, and organs of the body or worse still inducing terminal diseases in consumers when ingested as a flavour enhancer in food. Despite evidence of negative consumer response to monosodium glutamate, reputable international organizations and nutritionists have continued to endorse monosodium glutamate, and reiterated that monosodium glutamate has no adverse reactions in humans, (Okwuraine, 1992). The Food and Drug Administration (FDA) of the United States reports that monosodium glutamate is safe and that it should be maintained on the "General Recognized as Safe" (GRAS) - list of foods.

Monosodium glutamate is thus reportedly permitted as a safe food additive that needs no specified average daily intake or an upper limit intake requirement. National Agency for Food and Drug Administration Control (NAFDAC) has also expressed the view that monosodium glutamate is not injurious to health (Rogers and Blundell, 1990). The U.S. Food and Drug Administration (FDA) has admitted that processed free glutamic acid found in reaction flavours which are produced from a combination of specific amino reducing sugars, animals or vegetable fats or oils and optional ingredients including hydrolysed vegetable protein are carcinogenic (Food Chemical News, 1993). Technically, glutamate and monosodium glutamate are salts of glutamic acid (Olney, 1989).

In the sixties, the symptoms associated with monosodium glutamate were referred to as “Chinese Restaurant Syndrome”, as Chinese restaurants commonly added monosodium glutamate to their foods and many people felt the effect of it after dining. These symptoms ranged from burning sensation of different parts of the body, headache or numbness in the face to chest pain, nausea or vomiting (Kwok, 1968).

#### Objective of the research:

- To know whether or not monosodium glutamate is harmful for consumption
- To know at what concentration is the test material (monosodium glutamate) harmful

### MATERIALS AND METHODS

This research study was carried out between January to October 2010 in the Biochemistry Laboratory of the Department of Applied Sciences, Kaduna Polytechnic, Nigeria.

**Test materials:** Ajinomoto which is one of the trade names of the concentrated (99% pure MSG) forms of Monosodium Glutamate (Fig. 1) was procured from Central Market Kaduna, Nigeria. The test materials (MSG) was stored and protected from direct sunlight until time of drug administration. Appropriate volumes of the preparations were administered orally to the experimental animals via a syringe and canulla (1 mL capacity).

**Animals:** Adult Wister Albino Rats weighing (161.8-225.4 g) of either sex was used for the Nephrotoxicity and Hepatotoxicity studies. The toxicity tests were also sub-chronic. The animals were procured from pharmacology sciences department’s animal house, Ahmadu Bello University, Zaria, Nigeria. They were fed *ad libitum* with a mixture of poultry grower’s mash and corn pellets procured from Grand Feeds Limited, Jos-Nigeria and had free access to water (Kaduna Polytechnic Water Supply).

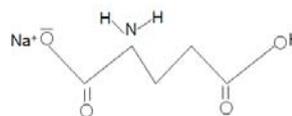


Fig. 1: Chemical structure of Monosodium Glutamate (MSG)

The animals were maintained under the same condition of humidity, temperature and 12 h light/darkness cycle. The animals were acclimatized for 2 weeks before the commencement of the toxicity study.

**Chemicals:** Kits for Glutamate Oxaloacetate Transferase (GOT, AST), Glutamate Pyruvate Transaminas (GPT, ALT) Alkaline Phosphatase (ALP), Total and Conjugative Bilirubin, Urea, Creatinine Random Blood Sugar (RBS) and Cholesterol were used for the biochemical analysis of the serum samples.

**Sub-chronic toxicity studies:** Twenty (20) Wister albino rats were procured and then divided into four groups A, B, C and D of five animals each. The first group (A) served as control while the remaining groups B, C and D were given 200, 300 and 400 mg/kg body weight respectively of the test material (monosodium glutamate) for four (4) weeks. The first day of dosing was designated as Do, whereas the day of sacrifice as D<sub>28</sub>.

**Weekly body weight:** The weekly Body Weight (BW) of each animal was assessed using a sensitive electronic balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

**Mortality and clinical changes:** During the 4-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing up to 3 h after dosing.

**Preparation of sera samples:** On the 28<sup>th</sup> day (D<sub>28</sub>) of the dosing period, all the animals were sacrificed by bleeding using dissecting kit and blood samples collected in plastic test tube and allowed to stand for 3 h to ensure complete clotting. The clotted blood samples were then centrifuged at 300 rpm for 10 min with the clear serum samples aspirated off and stored frozen.

#### Serum biochemistry:

**Serum bilirubin:** Six test tubes were prepared and labeled as standard, standard-blank; test<sub>1</sub>, test-blank, test<sub>2</sub>, test-blank<sub>2</sub> (for total and direct bilirubin) respectively. Distilled water (2.7 mL) was taken into each tube. Serum and bilirubin standard (100 mL) were added to test<sub>1</sub>, test-blank, test<sub>2</sub>, test-blank<sub>2</sub>, and standard-blank, respectively. Mixed Diaazo B, (100 mL plus 10 mL of Diaazo A) was

also added to tests and standard. Methanol, 3.5 mL was added into test<sub>2</sub>, and test-blank<sub>2</sub> (for total bilirubin). The tubes were mixed and allowed to stand at room temperature for 15 min the tubes were read at 540nm. The result of serum bilirubin was obtained using the following expression:

$$T_1 - TB_1 \times 8.3 \text{ umol/L/S} - SB \text{ for direct bilirubin}$$

where;

T<sub>1</sub> = Test

TB<sub>1</sub> = Test - blank

S = Standard

and

SB = Standard Blank

$$T_2 - TB_2 \times 16.2 \text{ mmol/L/S} - SB \text{ for total bilirubin}$$

where;

T<sub>2</sub> = Test 2

TB<sub>2</sub> = Test - Blank<sub>2</sub>

S = Standard

**Serum GPT (ALT):** One test tube was prepared and labeled as test. Into the test tube, 1000 mL of working reagent was pipetted. Sample, 100 mL was added into the tube. The contents were mixed and incubated at 37°C for 1 min. The change in absorbance per minute (OD/min) during 3 min was measured. The result was obtained using the following expression:

$$\text{AST activities (I.u)} = \text{OD/Min} \times 1768$$

Where OD = Optical density

**Serum GOT (AST):** One test tube was prepared and labeled as test. Into the test tube, 100 mL of working reagent was pipetted. Sample, 100 mL was added into the tube. The contents were mixed and incubated at 37°C for 1 min. The change in absorbance per min (OD/min) during 3 min was measured. The result was obtained using the following expression.

$$\text{AST activity (1 m)} = \text{OD/min} \times 1768.$$

Where OD = optical density

**Serum ALP:** Four (4) clean test tubes were labeled as test, test-blank, standard and standard blank. To the test tube labeled test, 1 mL of alkaline buffer was taken and 1mL of phenylphosphate substrate was added. These were incubated at 37°C for a minute. Serum, 0.1 mL was added into the tube in the incubator. These were mixed and incubated at 37°C for 5 min. The test tubes were removed and sodium hydroxide (0.5 N), 0.8 mL was added. Sodium bicarbonate (0.5 N), 1.2 mL was added. Para-(4)-amino antipyrine, 1.0 mL was added and finally 1.0 mL

potassium ferricyanide was added. These were mixed and read at 510 nm after zeroing the instrument with blank. Test blanks were treated as test but serum was added after adding the sodium hydroxide. To the test tube labeled standard, 1.2 mL of buffer was pipetted. Phenol, 1.0 mL was added and was treated as test after incubation. To the test labeled blank, 1.2 mL of buffer was taken. Water, 1.0 mL was added and was treated as test after incubation. Serum alkaline phosphatase activity was obtained using the following expression:

$$T - TB / S - SB \times 71 \text{ m/L}$$

where;

T = Test, TB = Test Blank, S = Standard and SB = Standard Blank

**Serum chloride:** To 1.8 mL deionized distilled water, 0.2 mL of serum was added, two drops of the indicator diphenyl/carbozone was added. The beaker was shaken to mix and the content was titrated with mercuric nitrate solution from a 2.0 mL pipette (graduated in 0.01 mL).

**Calculation:**

chloride: mmol/L = volume of mercuric nitrate used × 100.

**Serum cholesterol:** Clean test tubes were labeled test, standard and blank. 10 mL of distilled water was added to the blank test tube, 10mL of standard was added to the test tube labeled standard while 10mL of sample was added to test tube labeled test. Reagent 1 was then added to the test standard and blank (1000 mL each). The mixture was then mixed and incubated for 10 min at room temperature. The absorbance of test was then measured against reagent blank within 60 min.

**Calculation:**

Concentration of cholesterol in sample =

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{concentration of standard Normal value (2.5-6.5)}$$

**Serum creatinine:** Test tubes (4) were arranged in rack, 1.5 mL of distilled water was pipetted into the precipitating tubes, 0.5mL of the test serum was then added to the precipitating tubes, after which 0.5 mL sulphuric acid was added and followed by 0.5 mL of sodium tungstate. The contents were then mixed thoroughly, centrifuged at 300 rpm for 5 min 1.5 mL of the supernatant was transferred with a micropipette tube into a clean and dry grease free glass test tube, 0.5 mL 0.75% NaOH added, followed by 0.5 mL picric acid., contents mixed thoroughly incubated at room temperature for 15 min and mixture was read spectrophotometrically at 520 nm.

The values of the measured serum creatinine were deduced using the formula:

$$X = T/s \times C$$

where;

T = Test serum sepectrophoto-metric value = x

S = Standard (normal) serum value = 45-47

C = Concentration of standard = 530

**Reference values and normal range:** The normal range for the test of creatinine ranges from 72-126 mmol/L (08-1.4 mg %) in serum.

**Serum Urea:** 100 mL of the test serum was accurately dispensed into a clean and dry universal bottle which 10 mL distilled water was added bringing the dilution to 1:100, 1 mL of the resulting mixture was then withdrawn into a clean test tube 1 mL distilled water added, 2 mL of urea was then added, followed by 2 mL of the urea colour reagent, preparation was mixed thoroughly, incubated at 100°C for 20 min, then cooled in a water bath at room temperature for 10 min and absorbance read spectrophoto metrically at 520 nm: The values of the measured serum urea were deduced using the formula:

$$X = T/s \times C_s$$

where;

T = Test serum sepectrophoto-metric value

S = Standard (Normal) serum level

Cs = Concentration of the standard = 10

**Serum Glucose:** Three test tubes were prepared and labeled as blank, test and standard. Phenol (4 mL) was pipetted into each tube, distilled water, serum and glucose (standard (50 mL) were added into tubes 1 (blank), 2 (test) and 3 (standard), respectively.

Glucose colour reagent, 1mL was added into each tube. The contents were mixed and incubated at 37°C for 20 min and read at 510 nm using the blank to set the instrument to zero. The results were obtained as follows:

Serum glucose (mmol/L) = OD Test /OD Standard  
 × Concentration of Standard (10 mmol/L)  
 where; OD = Optical Density

## RESULTS

**Effect of MSG on the body weight of treated rats:** Result indicated progressive increase in the body weight generally irrespective of the group and MSG concentration administered. No significant difference in growth proportion within the groups between the week before treatment and first week of treatment.

A tremendous significant difference in weight gain between the control group (1.2%) and the treated group with % weight gains 1.7, 3.5 and 1.2%, respectively was observed.

The weight gain is proportional to the concentration of MSG administered. (Table 3).

**Effect of MSG on Hepatic function indices:** The effect of the MSG on the liver function test parameters are

Table 1: Effects of MSG on some plasma biochemical parameters for kidney function assessment of rats on termination of treatment

Biochemical parameters reference values	Treatment (mg/kg body weight)			
	Control	200	300	400
Urea (mmol/L) 3.0-6.6	5.88±0.28	5.30±0.95	7.92±51	7.36±4.36
Creatinine (mmol/L) 72.0-125	123.75±2.63	119.80±25.7	207.0±115.01	204.4±100.4
Sodium (Na) (mmol/L) 135-150	138.0±2.50	138.40±3.20	137.60±3.21	137.60±2.92
Potassium (K) (mmol/L)	5.55±0.90	5.40±0.39	6.52±0.96	5.92±0.45
Chloride (Cl) (mmol/L) 97-108	98.50±4.12	98.80±5.76	96.40±5.90	95.60±4.98

Values are expressed as mean±SD for N = 5; MSG: Monosodium Glutamate

Table 2: Effects of MSG on some plasma biochemical parameters for liver function assessment of rats on termination of treatment

Biochemical parameters Reference values	Treatment (mg/kg body weight)			
	Control	200	300	400
RBS (mmol/L)	6.35±1.39	7.94±4.73	10.06±6.25	15.52±0.98
GOT (mL/mg) (AST) 45.7-80.8	21.75±0.50	221.40±45.99	251.40±52.43	230.60±89.02
GPT(mL/mg) ALT 1.5-30.20	39.25±0.50	96.40±16.56	115.60±54.28	117.60±63.97
Cholesterol 10-54 mg/dL	1.30±0.23	1.26±0.22	1.26±0.22	1.62±0.48
ALP (mL/mg) 56.8-128	90.0±1.6	3138.80±29.4	2202.20±86.32	200.20±57.75

Values are expressed as mean±SD for N = 5; MSG: Monosodium Glutamate; GOT: Glutamate oxaloacetate transaminase; RBS: Random Blood Sugar; ALP: Alkaline Phosphatase

Table 3: Effect of MSG on the body of the rats treated and % wt gain

Group	B/AF	WK1	WK2	WK3	WK4	Wt gain (%)
(mgkgwt)	160.7±	161.8±	165.1±	166.6±	166.0±	1.2
B(200mg/kg)	170.6±	173.2±	178.3±	180.4±	182.4±	1.7
C(300)	183.4±	188.4±	192.8±	201.3±	210.6±	3.5
D(400)	220.5±	225.4±	228.2±	234.0±	236.6±	

Values are expressed in mean±SD for N = 5; B/AF = Before Animal Treatment with MSG wk = week; wt gain (%) = Percentage weight gain

presented in Table 2. Which indicate elevation in GOT, GPT ALP and RBS levels when compared to the control group. The elevation in all cases is dose dependant.

The increase in concentration of the parameters significantly differ from the control. No significant difference in cholesterol level except at 400 mg/kg body suggesting dose weight which value increased dependant effect.

Effect of MSG on kidney function parameters Table 1 shows the mean Kidney function values of the rats treated with MSG. from the table urea and creatinine levels were significantly increased ( $p < 0.05$ ) when compared with the control and above the normal reference values. No significant variation were obtained in the serum electrolytes tested when compared with control and all were within the normal reference values.

## DISCUSSION

LD<sub>50</sub> of MSG in the literature was found to be 19.9 g/kg as reported by (IFC, 1987). However, it is widely criticized as a parameter for toxicity assessment (Temlsnel, 2002) But even so, it gives clue on the range of doses that could be used in subsequent toxicity testing and further revealed the possible clinical signs elicited by the particular compound investigated (Aniagu *et al.*, 2004). This present research looked into the sub-chronic effect of MSG on the experimental Animals (Rat) with special emphasis on the Renal and Hepatic toxicity.

Ordinarily Hepatic cell damage is characterized by a rise in plasma enzymes such as Aspartate. Transaminase (Glutamate Oxaloacetate Transaminase SGOT), Glutamate Pyruvate Transaminase (GPT) also term ALT (Alanine Transaminase), Alkaline Phosphatase (ALP) and Lactose Dehydrogenase (LDH).

This investigation revealed that SGOT concentration were consistently higher than that of the SGPT level which is expected, since the ICF contain more of SGOT than SGPT. The release of SGOT therefore is slow due to its higher concentration in different tissues like liver, kidneys, heart and pancreas (Aniagu *et al.*, 2004). It is important to note that ALT is a sensitive marker enzyme in cytosol of hepatocytes and it's level within limits is indicative of hepato-cellular damage and that can therefore provide quantitative assessment of the degree of damage sustained by the liver (Aniagu *et al.*, 2004).

Hence the elevated level of ALT at 200, 300 and 400 mg/mg body weight could be an indication of hepatocellular damage induced by MSG. However, this can be better substantiated if further studies on the hepatic cell histology is conducted. So Hepatotoxicity study to involve organ histology would allow better assessment of MSG effect on liver cells and therefore, recommended. Cholestatic liver disease is characterized by increased

level of ALP (Kaneko, 1989) coupled with high level of bilirubin (Mayne, 1996). The trend of ALP significantly increase gave an indication that the hepatic capacity of the liver is grossly affected by MSG. Higher level of glucose (Table 2) showed the tendency of MSG to induced hyperglycemic effect therefore caution and close monitoring of the glucose level might be necessary.

Variation in cholesterol level was insignificant within the groups, however, groups treated with highest close (400 mg/kg body weight had shown elevated cholesterol level which suggest possible interference with fat metabolism. Earlier report by Kawakita *et al.*, 2005 that the potential explanation for MSG-obesity link lies in the alteration of regulatory mechanism that affect fat metabolism. Further finding is highly recommended to rule out the possible interference since, Table 3 indicated that diet were well accepted by the treated rats, suggestion no possible cause of any alteration in carbohydrate, or fat metabolism in these experimental animals. Hence no adverse interference with the nutritional benefit i.e., weight gain expected of animal on normal ration and water supply (*ad libitum*)

This investigation showed a remarkable elevated level of urea and creatinine within the MSG treated Rats when compared with control. Serum urea and creatinine increases as the ability of the kidney to filter fluid within the body declines. In a very general terms a rising level of creatinine significance an increasing problem with poorly performing kidneys (Edwards, 1991; Cheesbrough, 1991). Hence there is possible link between MSG and Renal impairment. However, no significant variation recorded between the electrolytes of the MSG treated Rats and controls and the values were within the normal reference value, which indicate normal renal capacity to excrete the electrolyte which might possibly reflect the preservation to some extent of the renal integrity.

## CONCLUSION

This study could be regarded as a preliminary research findings, which necessitate further investigation to establish the mechanism of toxicity of the compounds on the studied organs. Prospective studies should consider tissue histology, aspect of reproductive and genetic toxicity, mutagenicity and carcinogenicity effects. Nevertheless findings have shown that the granural study is significant and that Monosodium Glutamate is likely to produce severe toxicological effects on liver and kidney when consumed in high doses.

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