

1 **Severity of monosodium glutamate in liver of sprague-dawley rats**

2 **Running Headline:** Severity of MSG in rats liver

3 **ABSTRACT**

4 The safety of monosodium glutamate usage has generated much controversy, due to the
5 adverse reactions and toxicity of monosodium glutamate (MSG) which is commonly used as
6 a food additive and there is growing concern that this may have a toxic effect on many body
7 organs. The effect of analar and food grade monosodium glutamate (MSG) on the liver of
8 Sprague-Dawley rats was studied. Fifty female Sprague-dawley rats with an average weight
9 of about 100 – 150g were randomly assigned into 5 groups A, B, C, D and E of 10 rats each.
10 The treatment groups (A, B and C) were given 0.10g/kg, 0.15g/kg and 0.20g/kg body weight
11 of analar grade monosodium glutamate while treatment group D was given 0.20g/kg body
12 weight of commercial food grade monosodium glutamate in 1.5ml of water, respectively on
13 a daily basis. The control group (E) received equal amount of water without monosodium
14 glutamate for fourteen days. The rats were sacrificed on day fifteen of the experiment. The
15 liver of the rats were carefully dissected and quickly fixed in 10% formal saline for routine
16 histological procedures. Significant body weight increase was noted in MSG treated rats and
17 the histological findings in the treated groups showed evidence of hydropic degeneration of
18 hepatocytes, periportal cellular infiltration by mononuclear cells, severe portal and central
19 venous congestion with more severe changes in the group that received 0.20g/kg of analar
20 MSG. These findings indicate that MSG induced considerable structural changes in the liver
21 of Sprague-dawley rats; these changes are more severe at higher doses which may affect
22 liver function.

23 **Keywords:** Monosodium glutamate; histolopathology; liver; rats; hepatocytes

24 **INTRODUCTION**

25 Monosodium glutamate (MSG), also known as sodium glutamate is the sodium salt of
26 glutamic acid, one of the most abundant naturally-occurring non-essential amino acids
27 (Ninomiya, 1998). The commercial-grade is sold in most open market stalls and stores in
28 Nigeria as “Ajinomoto”. MSG is found in tomatoes, Parmesan, potatoes, mushrooms, and
29 other vegetables and fruits (Specter, 2014). MSG is used in the food industry as a flavour
30 enhancer with an umami taste that intensifies the meaty, savory flavour of food, as naturally
31 occurring glutamate does in foods such as stews and meat soups (Ikeda, 2002).

32 In Nigeria, most communities and individuals often use MSG as a bleaching agent for
33 the removal of stains from cloth. Though MSG improves taste stimulation and enhances
34 appetite, reports indicate that it is toxic to human and experimental animals (Belluardo *et al.*,

1990). Ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Geha *et al.*, 2001). MSG has a toxic effect on the testis by causing a significant oligozoospermia and increase abnormal sperm morphology in a dose-dependent fashion in male wistar rats (Onakewhor *et al.*, 1998). It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Oforofuo *et al.*, 1997). MSG has a toxic effect on many body organs by altering ionic permeability of neural membrane and induces persistent depolarization (Robinson, 2006). It has been reported that MSG has neurotoxic effects resulting order and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Al-zheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (Samuels, 1999).

The liver is the largest glandular organ of the body, weighing between 1.4-1.6kg (Johnson, 1995). It lies below the diaphragm in the thoracic region of the abdomen. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, production of bile; an alkaline compound which aids in digestion, and detoxification of most substances (Gartner *et al.*, 2000). Since the liver is involved in the performance of these varied functions, it may be susceptible to injury resulting from toxic substances. Therefore, the present study is aimed at studying the severity of monosodium glutamate using its analar and commercial food grade on the liver of young female rats.

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MATERIALS AND METHOD

Chemicals

The chemicals used in this study were an analar grade MSG ($C_5H_8NNaO_4 \cdot H_2O$) and the commercially available food grade package sold in most open market in Nigeria. A stock solution was prepared by dissolving known gram (g) of both analar grade and food-grade monosodium glutamate crystals differently in separate bottles in known millilitres (mL) of distilled water. The dose scheduled was so adjusted that the amount of MSG administration per animal was as per their respective group weights.

Experimental Animals

Fifty (50) female Sprague-Dawley rats with an average weight of about 100 – 150g were used for the experiment. The rats were housed under appropriate controlled room in approved cages with the standard temperature ranging between $26.5 \pm 2^{\circ}C$ and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to humidity and were fed to a dry balanced meal (Grower' mash) for experimental animals, with a constant source of water. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards as well as ethical guidelines of animal care unit of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Clinical examination of the experimental animals

The animals were randomly divided into 5 groups of 10 rats each. Group A received 0.10g/kg body weight, Group B 0.15g/kg body weight, Group C 0.20g/kg body weight of analar grade MSG in 1.5ml of water daily while Group D received 0.20g/kg body weight of commercial

81 food grade MSG also in 1.5ml of water daily for fourteen days. Group E served as the control
82 and all grades of MSG were administered orally.

83 ***Biochemical Analysis of the serum***

84 At the end of treatment period, blood samples of the rats were taken through the
85 ocular route into glass EDTA bottle. Serum was separated by centrifugation at 3000rpm for
86 10 min. The serum was collected and used for analysis, which included total protein, blood
87 glucose, albumin, globulin and enzymes activities (AST and ALT).

88 ***Glucose and cholesterol assay***

89 Glucose was determined by spectrophotometric method as described by Teuscher and
90 Richterich (1971) and cholesterol was determined by enzymatic colorimetric reaction
91 according to Siedel *et al.* (1981).

92 ***Transaminases assay:***

93 ALT and AST was determined by spectrophotometric method as described by
94 Schmidt (1963) and Oladipo *et al.* (2014).

95 ***Total serum protein assay***

96 Total serum protein was determined using the biuret method as described by Reinold
97 (1953).

98 ***Albumin assay***

99 Albumin was determined using the BGG (Bromocresol green) method as described by
100 Peters *et al.* (1982).

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102 ***Histopathological analysis of the ovaries***

103 At the end of the experimental period, the animals were sacrificed by cervical
104 dislocation and they were dissected, the livers were removed and fixed in 10% formalin

solution for routine histological techniques. The tissue pieces were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in melted paraffin wax. The wax block is then cut on a microtome to yield a thin slice of paraffin containing the tissue. The specimen slice is then applied to a microscope slide, air dried, and heated to cause the specimen to adhere to the glass slide. Residual paraffin was then dissolved followed by rinsing with an acid-alcohol and by rinsing with water to remove the acid-alcohol. The sections were stained with hematoxylin and eosin, and assessed under an Olympus microscope (Olympus Optical Co., GMBH, Hamburg, Germany). Images were captured using Camedia software (E20P 5.0 Megapixel;Hamburg, Germany) at 20X magnification

Statistical Analysis

The data gathered were processed using SPSS 17.0 software. Comparison between groups was made using One-way Analysis of variance (ANOVA).

RESULTS

Increased appetite and thirst was observed in the rat groups administered with MSG (both analytical and commercial grade) when compared to the control group. Body weight of the experimental animals was taken on weekly basis for two weeks. All groups gained weight but Group C (group treated with 0.20g/kg body weight of analar grade MSG) had highest weight gained after the fourteens days of MSG treatment.

Biochemical analysis of the blood serum showed that analar and food grade MSG administration increased aspartate aminotransferase, alanine aminotransferase, albumin, total protein and globulin levels when compared with the control as shown in Table 1. MSG administration also led to a decrease in glucose levels in the sera of all the treated groups. Significant decrease was recorded in the cholesterol levels of groups A, B and D except group C (Table 1).

Histopathology of liver of animals administered with 0.1g/kg body weight of analar MSG revealed that there was a mild diffuse hydropic degeneration of hepatocytes. Moderate periportal cellular infiltration by mononuclear cells was seen in the group administered with 0.15g/kg body weight of analar MSG. Furthermore, the animals administered with 0.20g/kg body weight of analar MSG showed severe portal and central venous congestion while animals administered with 0.20g/kg body weight of food grade MSG showed a mild periportal cellular infiltration when compared with the control group as shown in Figure 1.

Table 1: Biochemical analysis of the blood serum of experimental animals

Parameters	A	B	C	D	E
AST	1.63 ± 0.41^a	1.66 ± 0.19^a	1.74 ± 0.18^a	1.78 ± 0.16^a	1.51 ± 0.10^a
ALT	0.47 ± 0.06^a	0.47 ± 0.02^a	0.49 ± 0.07^a	$0.51 \pm 0.07^{a,b}$	0.38 ± 0.07^b
ALB	$6.23 \pm 0.22^{a,b}$	$6.67 \pm 0.39^{b,c}$	6.73 ± 0.31^c	$6.59 \pm 0.18^{a,b,c}$	6.12 ± 0.49^a
TP	$84.75 \pm 4.88^{b,c}$	$84.96 \pm 9.45^{b,c}$	86.94 ± 2.11^c	$76.42 \pm 5.31^{a,b}$	69.60 ± 8.75^a
GLOB	$45.82 \pm 7.93^{b,c}$	$47.44 \pm 3.95^{b,c}$	48.65 ± 5.28^c	37.82 ± 5.41^a	34.25 ± 10.98^a
CHOL	$77.18 \pm 16.36^{b,c}$	$79.93 \pm 7.88^{a,b,c}$	94.74 ± 11.31^c	64.66 ± 2.24^a	$87.10 \pm 11.97^{b,c}$
GLU	4.47 ± 0.94^a	4.77 ± 0.96^a	5.21 ± 0.28^a	5.26 ± 1.72^a	5.41 ± 0.79^a
BIL	7.29 ± 2.82^a	8.65 ± 1.46^a	9.86 ± 7.84^a	6.71 ± 1.95^a	6.90 ± 0.91^a

Each value is a mean of 3 replicates $\pm$ standard deviation; values accompanied by identical superscript letters are not significantly different ($p \leq 0.05$). Aspartate aminotransferase (AST- μ Kat/L); Alanine aminotransferase (ALT- μ Kat/L); Albumin (ALB- μ mol/L); Total protein (TP-g/L); Globulin (GLOB-g/L); Cholesterol (CHOL-mg/dL), Glucose (GLU- mmol/L); Bilirubin (BIL- μ mol/L). A = Group administered with 0.10g/kg MSG; B = Group administered with 0.15g/kg MSG; C = Group administered with 0.20g/kg MSG; D = Group administered with 0.20g/kg Food grade MSG; E = Control group.

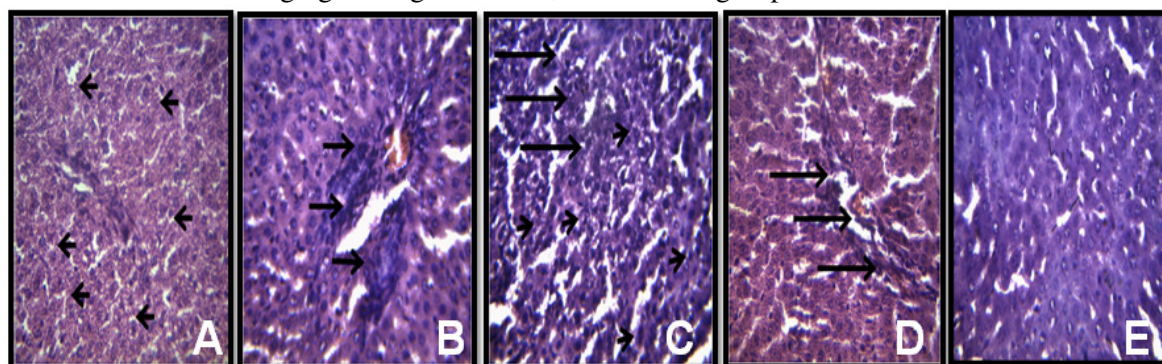


Figure 1: Histology of the livers of experimental rats after MSG treatment. A = Group administered with 0.10g/kg MSG; B = Group administered with 0.15g/kg MSG; C = Group

147 administered with 0.20g/kg MSG; D = Group administered with 0.20g/kg Food grade MSG;
148 E = Control group.
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150 **DISCUSSION AND CONCLUSION**

151 Monosodium Glutamate (MSG) is one of the commonly and widely used food spices
152 in our daily diets (Walker and Lupien, 2000). Its consumption has increased world wide as
153 flavouring and food additive in our daily cooking to improve palatability and food preference
154 in a meal (Chaudari and Roper, 1998). However, consumption of this food additive has been
155 shown to cause metabolic disorders and oxidative damage of tissues (Dinz *et al.*, 2005) which
156 may possibly be responsible for the pathophysiology of many diseases like cancer, diabetes,
157 endothelial dysfunction brain lesion and Coronary Heart Disease (Dinz *et al.*, 2005; Mallick,
158 2007).

159 After administration of analar and food grade MSG, the rats showed significant
160 increase in body weight, this agrees with the previous work of Oladipo *et al.* (2015) and
161 Oluba *et al.* (2011) who reported that consumption of MSG increases body weight gain.
162 There was a significant increase in the activity levels of the aspartate amino transferase,
163 alanine amino transferase, albumin, total protein, globulin and bilirubin dose-dependently in
164 the serum of the rats administered with MSG when compared to the control group. The
165 transaminases are abundant in the liver and are released into the blood stream following
166 hepatocellular damage, making them sensitive marker of liver damage (Al-Mamary, 2002).
167 The marked increase in the plasma ALT and AST activities observed in the monosodium
168 glutamate fed rats might be indicative of liver damage. This is in agreement with the
169 significant elevation of the serum marker recorded by Ajibade *et al.* (2013) which was also
170 confirmed by Nayira *et al.* (2009) and Oladipo *et al.* (2015) who reported a significant
171 increase in AST and ALT level upon MSG administration. Decrease in the activity of glucose
172 in MSG administered rats is suggestive of decrease glucose utilization through the glycolytic

pathway (Diniz *et al.*, 2004). Diniz *et al.* (2004) had suggested a possible deterioration of glucose tolerance in rats following administration. The reduced glucose tolerance could be attributed to decreased cellular sensitivity even under conditions of hyperinsulinemia observed in animals administered with MSG (Macho *et al.*, 2000).

In this study, liver sections of rats treated with low dose of analar MSG showed considerable structural changes, amongst which was mild diffuse hydropic degeneration of hepatocytes. This is in alignment with the previous work of Singh (1997) who reported the distortion and dilatation of the hepatocytes and their central vein. He further reported that the haematopoietic function of the liver may have been highly affected as a result of probable toxic effect of MSG. As reported by Singh (1997), MSG may have acted as toxins to the hepatocytes, thereby affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis. The hydropic and degenerative changes observed in this experiment may have been caused by the cytotoxic effect of MSG on the liver; which is in agreement with Waters *et al.* (1994).

The liver section of the rats treated with food grade MSG showed a mild periportal cellular infiltration by mononuclear cells. This is supported by Butler (1996) who reported hepatocellular necrosis in the periportal areas and fibroplasia with mononuclear leucocytic infiltration. The observed necrosis might lead to hepatitis as reported by Nwaopara *et al.* (2007) and the infiltration of mononuclear leucocytes into the portal area was probably an adaptive cellular response to possible hepatitis resulting from the toxic effects of MSG.

Furthermore, the animals administered with high dose of analar MSG showed severe portal and central venous congestion. Seeto *et al.* (2000) stated that liver failure had severe underlying cardiac disease that had often led to passive congestion of the liver. Also, Eweka *et al.* (2007) revealed that with increasing dose of monosodium glutamate consumption; there was varying degrees of dilatations of the central vein of the liver which contain lysed red

blood cells. This suggests that the distortion of the cyto-architecture of the liver could be associated with functional changes that may be detrimental to the health of the rats.

Conclusively, these findings showed that monosodium glutamate consumption may have some deleterious effects in the liver of adult Sprague dawley rats at higher doses and by extension may affect the functions of the liver. The results of the present investigation have shown that mono-sodium glutamate (MSG) is capable of producing alterations in the body weight and liver functions. These alterations appear in the liver probably because it is mainly responsible for detoxification of foreign compounds in the body.

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