Toxic Effect of High Doses of Monosodium Glutamate on the Kidney Histology of Adult Wistar Rat

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Abstract
Background: *Monosodium glutamate (MSG) is a flavour enhancer used extensively in the food industry, and in restaurants and homes. It is present in a wide variety of processed foods.*
Aim: *The aim of this study is to determine the effect of oral administration of MSG on the body weight and kidney morphology in adult wistar rats using graded dose.*
Methods: *Twenty five rats of both sexes were divided into five groups with free access to feed and water ad libitum for a period of four weeks after two weeks of acclimatization. The test groups (B-E) received graded doses of MSG in the following order; B-20mg, C-40mg, D-80mg, E-120mg and F-160mg/day for the period of four weeks. Experimental substance was prepared by dissolving 15.2grams of MSG salt crystals in 380mls of normal saline which was stored in a clean leak proof bottle and was kept in the refrigerator. The weight of the Wistar rats before and after acclimatization was recorded as well as the weight of the rats after each week of administration. The data gotten was analyzed using SPSS version 16.0.*
Result: *Kidney microanatomy in test group D and E compared to control shows degenerative changes in the form of interstitial congestion and necrosis. Test groups B and C showed no changes in kidney architecture as they appear normal and similar to the kidney architecture of the control group.*
Conclusion: *The study suggested that excessive consumption of MSG over time will adversely affect the kidney and further study from the data analysis showed no relationship between MSG and weight gain.*

Keywords: *Monosodium glutamate, Kidney, Rat, Effect, Excessive*

Introduction

Over time, because of man's inquisitive nature, food additives have been chemically processed and designed to play a very similar role to that of salt; of which monosodium glutamate is one of such additives. Monosodium glutamate (MSG) is a white crystalline

powder, it is the sodium salt of a naturally occurring non-essential amino acid, glutamic acid (Furst and Stehle 2004).

Monosodium glutamate is a flavour enhancer extensively used in the food industry, and in restaurants and homes. Several processed foods such as flavored chips and snacks, soups or sauces (canned, packed), prepared meals, frozen foods, marinated meats, fresh sausages, bottled soy or oriental sauces, and stuffed or seasoned chicken, manufactured meats, some hams, luncheon chicken and turkey, flavored tuna, vegetarian burgers and sausages are seasoned with MSG (Giacometti *et al.,* 1979). Glutamate also occurs naturally in various foods including cheeses, seafood, meat broths, poultry and vegetables. MSG poses no problem when present in small amounts in any food; the problem is maximized with cumulated small amounts which are in different common foods that are consumed daily. Because MSG has many aliases and titles it makes it very difficult to determine what foods contain this additive (Raben *et al.,* 2003).

Food flavoring has been shown to be necessary in elderly persons who have irreversible chemosensory deficit, Hence the need to improve the appetite of such individuals to ensure adequate dietary intake. Flavor enhancers are also used as replacement for salt in the management of conditions such as hypertension. Naturally, Glutamate is known to have an *umami* taste that makes food palatable and boost flavor acceptance (Fuke and Shimizu, 1993).

A study in1991 revealed that the average intake of MSG in United Kingdom was 580 mg/day for general population individual and 4.68 g/day for extreme users (Rhodes *et al*. 1991). The estimated average daily MSG intake per person in industrialized countries is 0.3–1.0g, but this is depended on the MSG content in foods and an individual's taste preferences (Geha *et al*., 2000). A combined inquiry by the governments of Australia and New Zealand in 2003 observed that a typical Chinese restaurant meal contains between 10 and1500 mg of MSG per 100 g (Freeman, 2006).

MSG has been categorized as a safe substance by the Food and Drug Administration (FDA) in 1959. A commissioned report by the FDA has shown that an unknown percentage of the population might react to MSG and develop MSG symptom complex. Reports from experimental studies have revealed that long term consumption of MSG resulted in a great number of toxic effects referred to as Chinese restaurant syndrome characterized by nausea, headache, sweating, chest tightness, and/or a burning sensation in the back of the neck (Raiten *et al.,* 1996), hyperphagia, asthma, obesity, memory impairment, and damage to hypothalamic neurons, hence large amounts of MSG in foods can be detrimental to health (Taliaferro, 1995).

The Kidney is a major excretory organ essential to the urinary system and also serve homeostatic functions such as the regulation of electrolytes (including salts), maintenance of acid–base balance, maintenance of fluid balance, and regulation of blood pressure (via the salt and water balance). They serve the body as a natural filter of the blood, and remove water-soluble wastes which are diverted to the bladder (Cotran *et al.,* 2006).

Several researchers have studied MSG over the years and they have revealed that MSG causes significant increase in weight gain as well as organs (Tawfik and Al-Badr, 2012), high blood pressure in Chinese ethnic minorities, increase in haemoglobin levels (Shi *et al*., 2010).The purpose of this research work is to further investigate the toxic effects of MSG on

kidney histology of Wistar rat and to determine if this effect is dosage dependent. It may possibly provide a baseline for the regulation of the intake of MSG.

Materials and Method

Research Design

Twenty five rats were used for the study and the experimental animals were divided into five groups (A-E) housed using five big cages elevated from the ground to prevent them from getting effected. Group A served as the control and received growers marsh and normal saline with no administration of the monosodium glutamate. Group B, C, D and E received graded doses of the monosodium glutamate (40mg, 80mg, 120mg and 160mg) prepared carefully to ascertain the concentration to the MSG to be administered for a period of 28days.

Study Area

This research was executed in Ekpoma, the administrative headquarters of Esan West Local Government Area, of Edo state, Nigeria.

Experimental Animal/ Housing Condition

Twenty five adult Wistar rats of both sexes of comparable sizes and weights ranging from 100-200grams were procured from the animal house of Basic Medical Science College of Medicine. The rats were acclimatized for two weeks (14days), kept in wire mesh cages elevated from the ground and the animal beds were changed weekly with fresh saw dust. During the acclimatization period, the rats were fed with grower's marsh and water ad libitum. The house was swept daily and disinfected weekends to prevent the rats from being infected in accordance with the standard guide for the care and use of laboratory animals.

Study Duration

The preliminary studies, animal acclimatization, ingredients procurement/preparation, administration period, processing, microscopy and evaluation of results lasted for a period of eight months (from February to October 2016).

Experimental Substance Preparation

A large quantity of MSG (trade name Ajino-moto) was procured from the royal market, Ekpoma. A large volume of experimental stock was prepared by dissolving 15.2grams of MSG salt crystals in 380mls of normal saline which was stored in a clean leak proof bottle. The stock was

Substance Administration

The administration of MSG was performed orally by using a 2ml syringe to transport the experimental substance through the oral cavity until it gets to the gut. This was done to monitor and control the quantity of MSG given to the rats unlike when mixed with their water. The weights of the rats were taken weekly to monitor the effects of the MSG on the rat's weights and relevant information was also documented.

Group A (control); received a total of4.8mls of normal saline per day (0.8mls/ rat)
Group B (40mg); received a total of 1.2mls of MSG per day (0.2ml/ rat)
Group C (80mg); received a total of 2.4mls of MSG per day (0.4ml/ rat)
Group D (120mg); received a total of 3.6mls of MSG per day (0.6ml/ rat)
Group E (160mg); received a total of 4.8mls of MSG per day (0.8ml/ rat)

Sample Collection and Analysis

Weight was measured before and after acclimatization, similar weight measurements were

done at the end of every week and average weight recorded accordingly. At the end of the administration period, the rats were rendered unconscious by using chloroform gas and the liver of each rat excised through a mid-line abdominal incision passing through the abdominal wall musculature into the peritoneal cavity. The excised kidney was described macroscopically, washed in cold saline, weighed and then fixed in 10% formol-saline for histological processing.

Histological Processing

Cut up Procedure

The excised organ was taken to the cut room for macroscopic examination were relevant information like the color, size (length, breadth and diameter), weight, cut surface was recorded and a laboratory number was generated for the tissue afterwards using the first alphabet of the kidney (k) and the group from which the liver was coming from. E.g. kA1, kA2, kB1, kC3, kD6, kE6 etc. Afterwards, the whole kidney organs of the experimental rats were dissected carefully; a small representative portion was removed and carefully placed in a tissue cassette carrying the new lab number. The cassette was completely immersed in 10% formol-saline and was sent to the laboratory for processing.

Automatic Tissue Processing

The tissues were processed using the automatic tissue processor according to the processing schedule used in the University of Benin Teaching Hospital (UBTH), Benin, Edo state, Nigeria. The plastic cassettes containing the tissue were processed by passing them through different baths as follows:

- 70% alcohol 2hours
- 80% alcohol 2hours
- 90% alcohol 2hours
- 90% alcohol 2hours
- 95% alcohol 2hours
- Absolute alcohol 2hours
- Xylene I 2hours
- Xylene II 2hours
- Molten paraffin wax I 2hours
- Molten paraffin wax II 2hours

After the last timing, the tissues were removed from their plastic cassettes and placed at the center of the metallic tissue mould after which, they were filled with molten paraffin wax 2-3°C above its melting point. Wax was allowed to cool, set slightly and was then put on ice to harden it for 15 minutes. The solidified tissue blocks were removed from their metallic case using a knife and after which excess wax was removed from the side of the block using the knife.

The blocks surface was carefully trimmed to expose the tissue and thereafter, serial sections of 3mm thick were cut using a rotary microtome. Tissue ribbons cut off was picked with a clean grease free slide and few drops of 20% alcohol was placed on the cut sections to help flatten and to remove minor folds from the tissue. The sections were floated in water bath at 55°C, picked up by the use of a clean frosted end slide and thereafter, the frosted end of the slide was carefully labeled with a 2B pencil for easy identification. The slide containing the cut sections were placed uppermost on a hot plate for 40minutes for adequate attachment of the sections to the slide and to also remove water from the slide. Finally, the already dried

slides were carefully arranged in the slide box ready for staining process.

Staining Procedure

Sections were stained with haematoxylin and eosin staining technique to demonstrate for general tissue structure

Principle

The principle states that haematoxylin being a basic stain will have affinity for the acidic component of the tissue while eosin being an acidic stain will have affinity for the basic component of the tissue.

Procedure

1. Sections were dewaxed in two changes of xylene 2minutes each
2. Sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%) 30 seconds each
3. Harris haematoxylin stain was applied to the section for 5 minutes
4. Sections were rinsed in water to remove excess stain
5. Sections were differentiated in 1% acid alcohol 3 seconds
6. Sections were blued in running tap water 10 minutes
7. Sections were counter stained with 1% eosin 2 minutes
8. Sections were finally rinsed in water, dehydrated in ascending grades of alcohol (70%, 80%, 95%, and absolute alcohol).
9. Sections were cleared in xylene, air-dried and mounted with dibutylphthalate propylene xylene (DPX).

The slides were examined under a light microscope and photomicrographs were taken.

Data Analysis

The obtained data were subjected to statistical analysis using SPSS (version 20). The test groups' values were compared with the values of the control group using ANOVA (Scheffe) at 95% level of confidence.

Result

From table 1, Test groups C, D and E presented signs of aggressiveness as part of their behavior and fecal nature (output, texture and quantity) were different in the entire groups as group C, D and E presented pale, gummy and semi-solid stools. On the other hand, there were no comparable changes in skin surfaces on the feet, hand, tail, mouth, ears and eyes. Tests groups C, D and E presented signs of aggressiveness of different levels. Three deaths were recorded in total during the course of this research; two before administration in group B and C and one death in group E during the second week of administration of which the cause of death was unknown and was not related to the dose administered. Both the control and test groups (B, C, D, and E) presented no observable changes in fur.

Table 1: Notable Physical Observations of Control and Test (Fed with Graded Dose of Monosodium Glutamate) Rats during the Experiment

OBSERVATIONS	CONTROL	GROUP B (40mg)	GROUP C (80mg)	GROUP D(120mg)	GROUP E(160mg)
Physical agility	Active	Active	Active	Very active	Very active
Behavioral changes (aggressive)	-	-	+	++	+++
Fecal nature (consistency)	Solid	Solid	Semi solid	Semi solid	Semi solid
Diarrhea	-	-	+	++	+
Water rejection	-	-	-	-	-
Birth	++	++	+++	++	+
Fur color	-	-	-	-	-
Death	-	+	+	-	+

Key:
+ = present in trace amount
++= present in moderate amount
+++= present in large amount
- =negative (absent)

Table 2 below, presented the body weight changes in the test and control groups. Although at every stage of the weight determination, the control group (group A) presented weight gain while the test groups (group B, C, D and E) presented a drop in weight. After acclimatization, body weights were found to increase linearly with highest increase observed in group E (240.00±41.83).

After the first week of administration, linear weight gain was observed in group A, B, C and D but a drop in weight was observed in group E (220.00±27.38).
At the second week of administration, weight gain was observed in the control and the weight across the tests groups remained constant but a small weight loss was observed in group B (255.00±27.38).
At the third week of administration, there was a slight fluctuation in weights across the control and the test groups.

Finally at the 4^{th} week of administration, weight gain was observed in the control (group A) and across the groups with a slight reduction observed this time in group C (245.00±11.18). Comparatively, these body weight variations at different weeks of the experiment were found to be significant only at the fourth week of administration with p value of 0.03.

Table 2: Showing the body weight changes of rats fed with MSG at various intervals

Weights (g)	CONTROL (Group A) (n=5)	Group B (n=5)	Group C (n=5)	Group D (n=5)	Group E (n=5)	P-Value
WBA	140.00±22.36	150.00±0.00	150.00±0.00	150.75±0.00	160.00±22.36	0.32
WAA	195.00±27.38	210.00±22.36	225.00±25.00	225.5±27.38	240.00±41.83	0.17
WK 1	270.00±27.38	260.00±13.39	250.00±35.35	240.50±54.77	220.00±27.38	1.74
WK 2	290.00±22.26	255.00±27.38	250.00±35.35	240.50±54.77	220.00±27.38	0.66
WK 3	280.00±27.38	250.00±0.00	255.00±20.91	260.25±37.91	250.00±35.35	0.43
WK 4	330.00±44.72	260.00±54.77	245.00±11.18	280.00±44.72	255.00±44.72	0.03
TOTAL	250.83±70.24	230.83±47.65	225.83±41.77	235.83±45.41	232.50±47.86	

KEY:
Significant: $p < 0.05$.
Not significant: $p > 0.05$
WBA: Weight before acclimatization
WAA: Weight after acclimatization
Values are mean ± Standard deviation
n: number of sample.

From table 3, the data analysis showed a significant increase in organ weight of test groups (B, C, D and E) who received 40mg, 80mg, 120mg and 160mg of MSG for a period of 28days compared to the control who received just normal saline. Kidney weight corresponds with the progressive increase in concentration of MSG.

Table 3: Showing the organ (kidney) weight changes of rats fed with MSG at various intervals

Parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
$\bar{X}$ ± SD	2.98 ±0.85	4.06 ± 1.04	4.60 ± 1.21	4.86 ± 0.97	5.16 ± 1.00
Number (n)	5	5	5	5	5

n; number of samples
P value <0.05 (Significant)

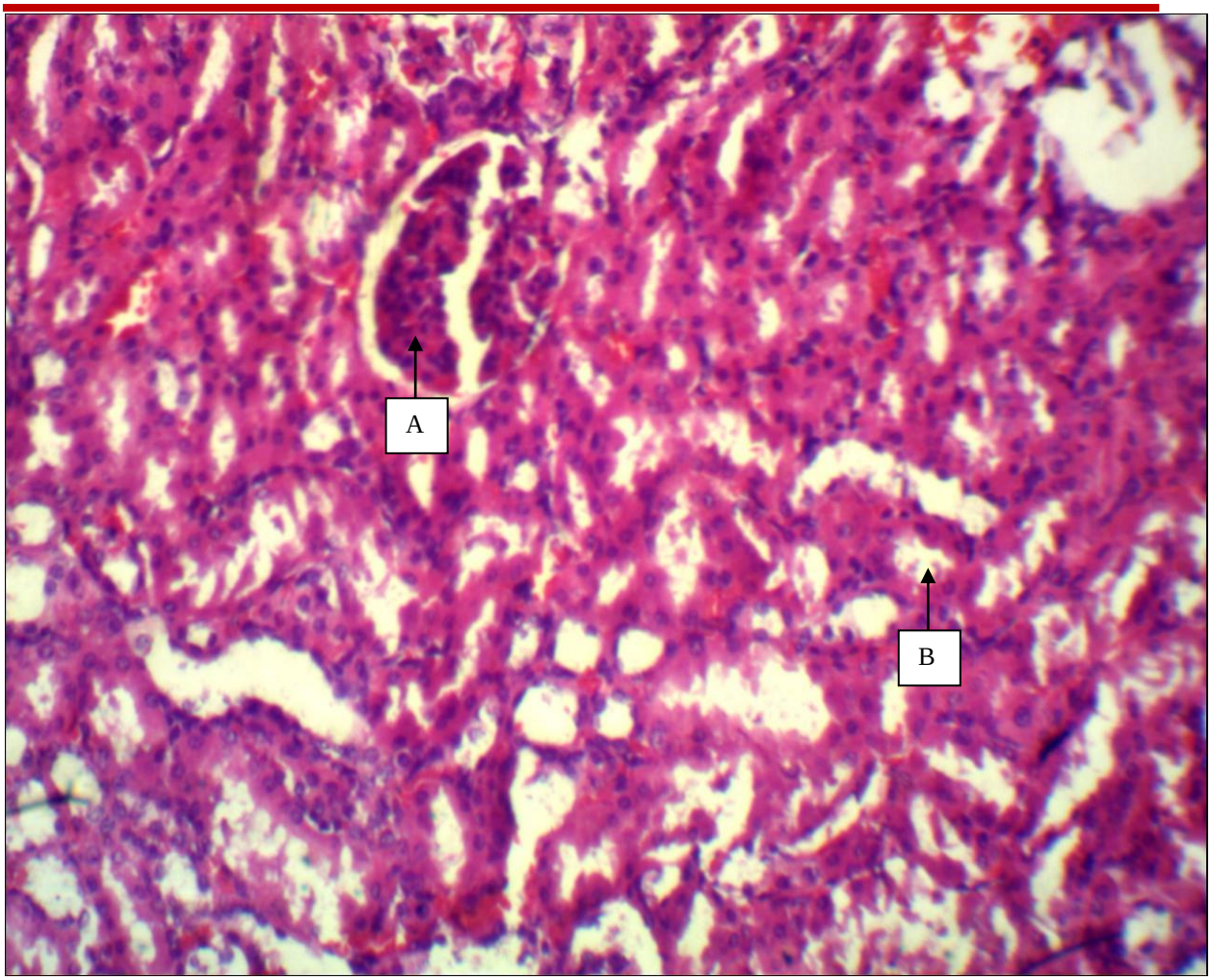

Fig 1 GROUP C; Rat kidney section (treated with 80mg monosodium glutamate) showing a normal histology composed of mainly renal corpuscles A and tubules B (H&E x 100)

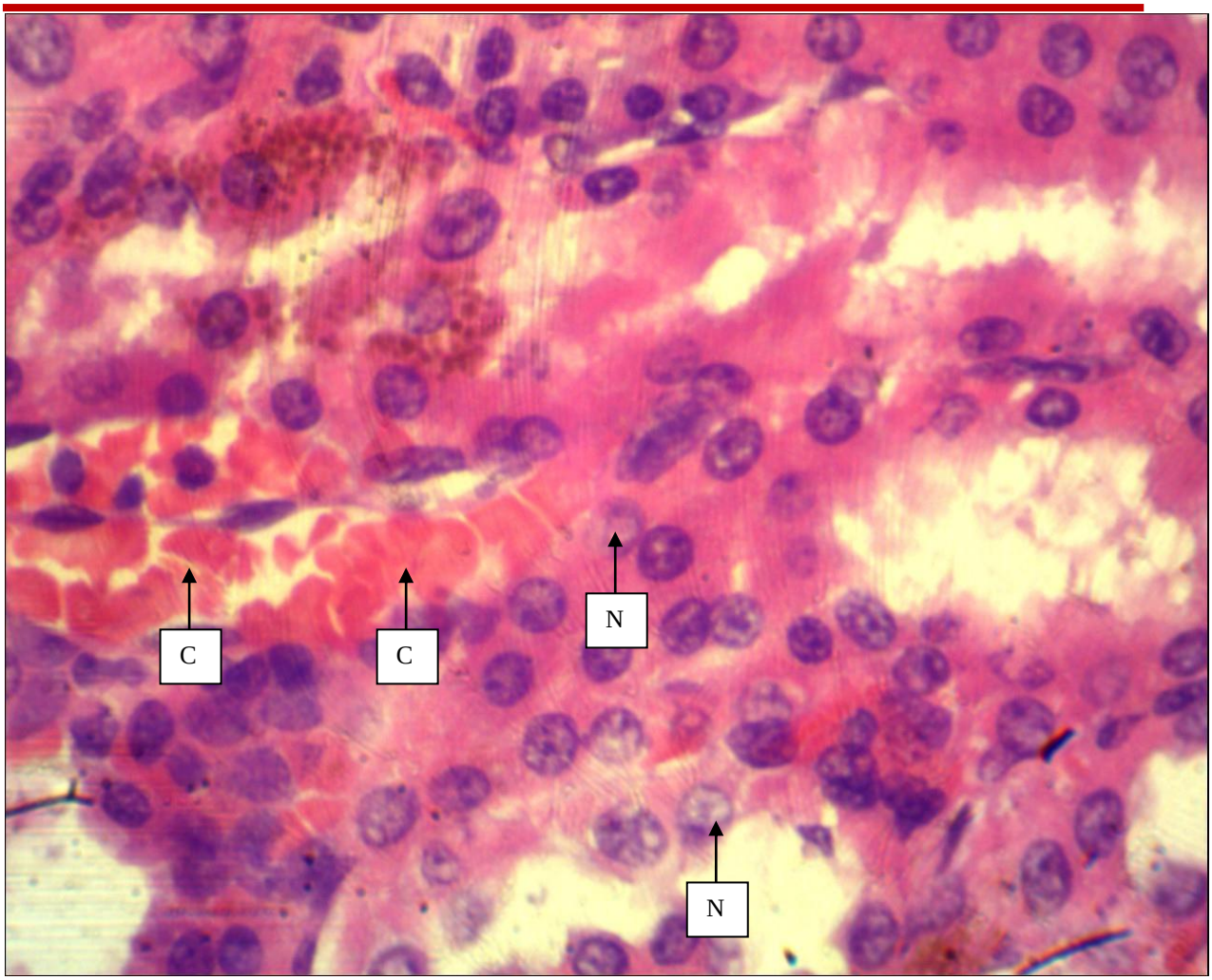

Fig 2: GROUP E: Rat kidney section (treated with 160mg monosodium glutamate) showing moderate interstitial congestion C and signs of necrosis N (H&E x 400)

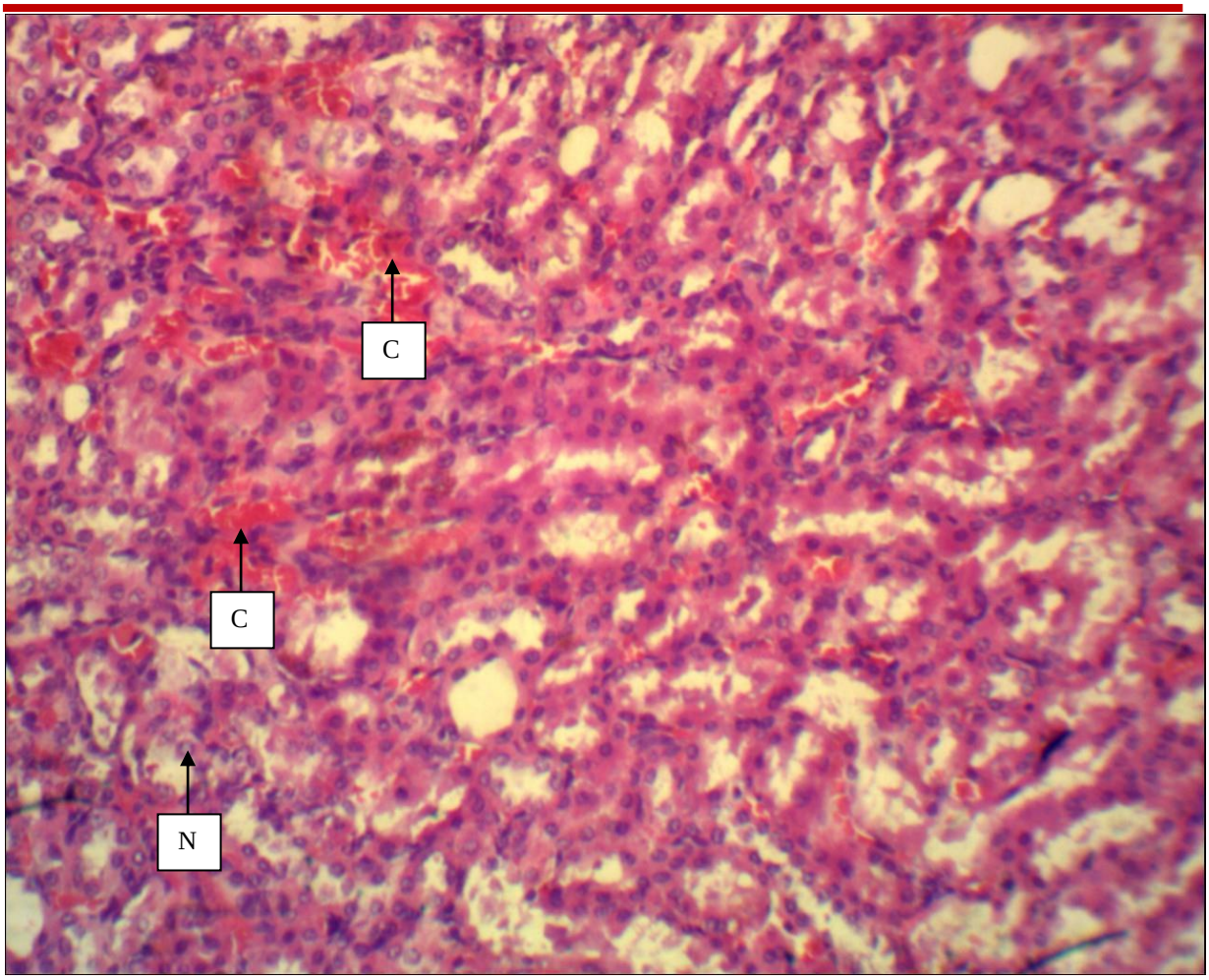

Fig 3: GROUP E: Rat kidney section (treated with 160mg monosodium glutamate) showing Moderate interstitial congestion C and signs of necrosis N (H&E x 100)

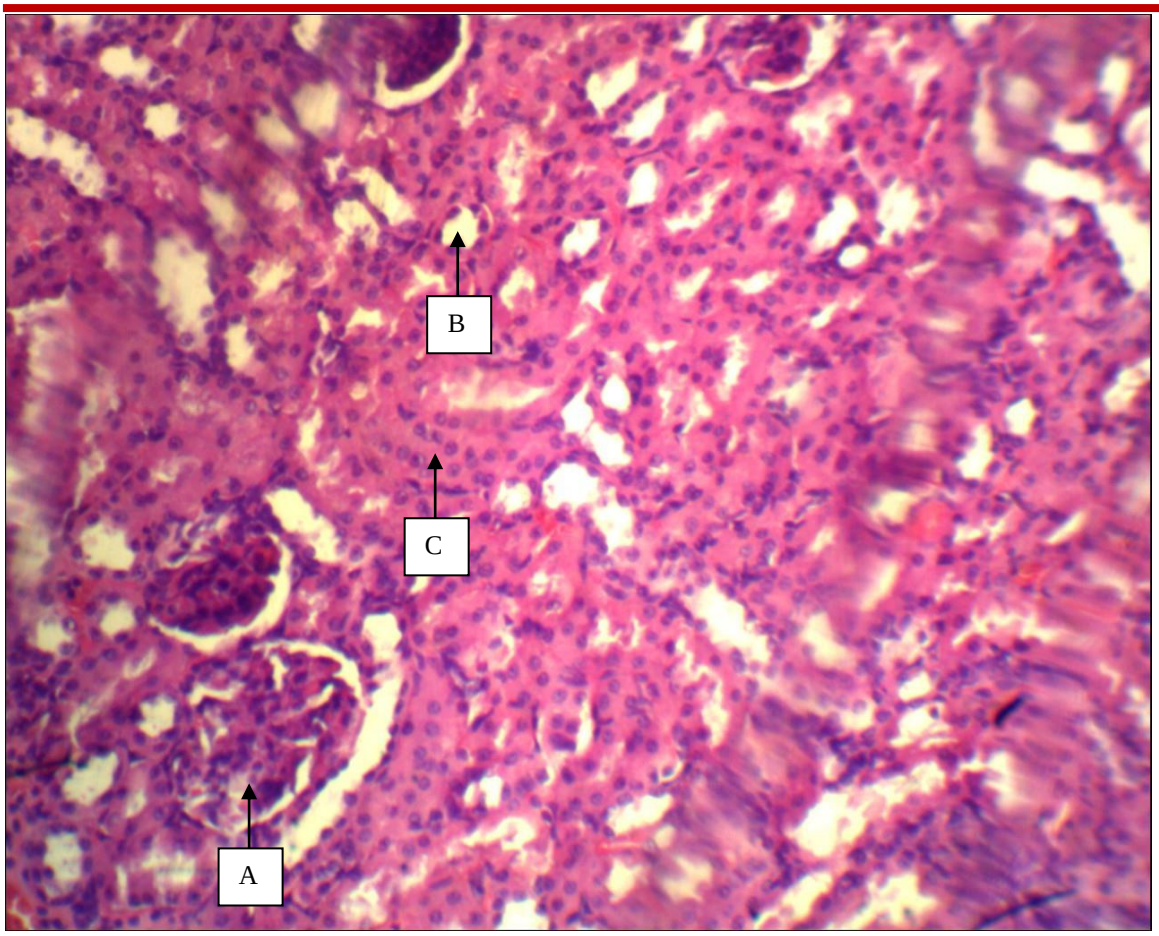

Fig 4: GROUP A; Control Rat kidney showing a normal histology composed of renal corpuscles A, tubules B and interstitium C (H&E x 100)

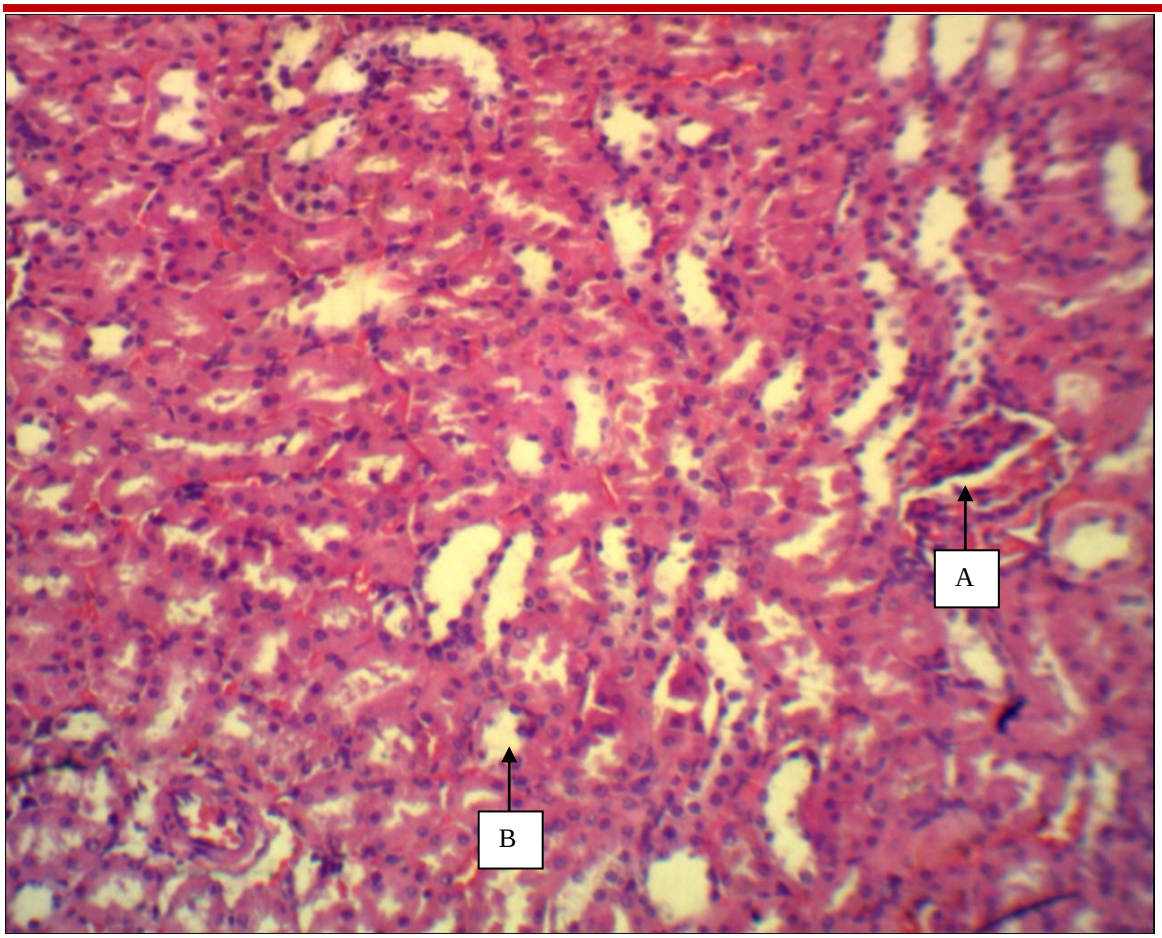

Fig 5: GROUP B; Rat kidney section (treated with 40mg monosodium glutamate) showing a normal histology composed of renal corpuscles A and tubules B (H&E x 100)

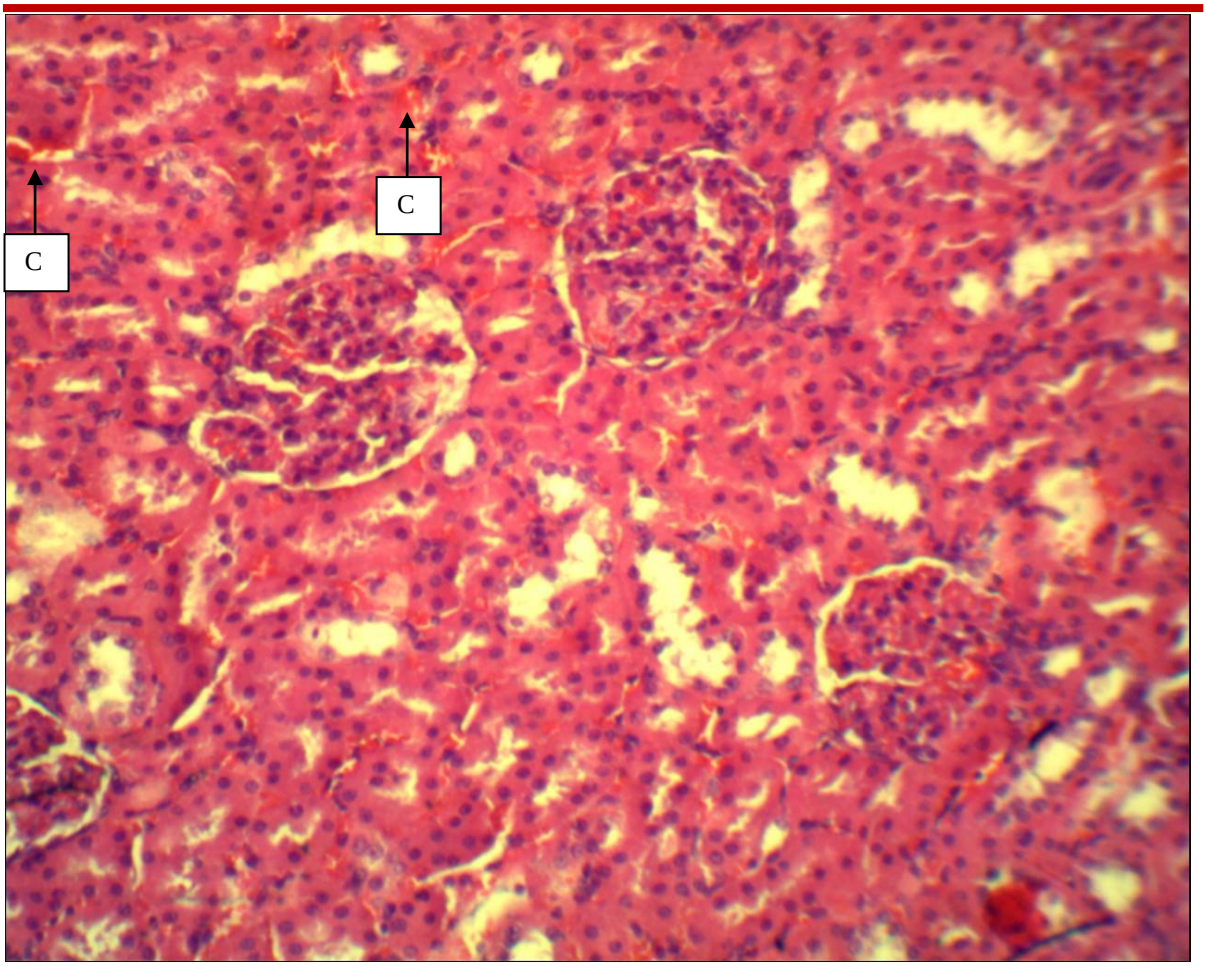

Fig 6: GROUP D; Rat kidney section (treated with 120mg monosodium glutamate) showing mild interstitial congestion C (H&E x 100)

Discussion

During the past three decades, there has been substantial controversy regarding the use of MSG in foods, at least in Western countries. The original source of this controversy appears to be a letter to the *New England Journal of Medicine* (Kwok, 1968) in which it was speculated that MSG (as one alternative among several other ingredients) could be the cause of adverse reactions following consumption of Chinese restaurant food. This article and subsequent publicity about MSG seems to have tapped into more general consumer concerns regarding food additives, resulting in an increasingly widespread belief among consumers that MSG is responsible for allergic reactions, variously asthma or "Chinese restaurant syndrome" of numbness, weakness, headaches and palpitations (Prescott and Young, 2002). Monosodium glutamate (MSG) is consumed in considerable amounts in almost all forms of food in Nigeria. The findings on body weight and histological changes are mostly in conformity with findings from previous studies.

Findings from this study showed reduction in the weight but food consumption continued to increase across the test groups. Fall in body weight was noticed in test groups (B-E) that received (40, 80, 120 and 160mg) concentrations of MSG compared to the control. The rats test groups (B, C, D and E) had a total weight average of 230.83±47.65, 225.83±41.77, 235.83±45.41 and 232.50±47.86 compared to that of the control (group A) 250.83±70.24 at the end of 28days.This finding is in agreement with the work of Shi *et al.*, (2010). Previous studies on experimental animals disagrees with the weight suppression

findings of this research and thus suggested a positive link between MSG and obesity; were weight gain was found to be significantly greater in MSG treated animals compared to the control. This could be as a result of appetite or even with consumption of similar amounts of food and improvement in the palatability of food by exerting a positive influence on the appetite centre (Hermanussen and Tresguerres, 2003; Hermanussen *et al.*, 2006).

The cytoarchitectural structure of the kidney of group B and C (40mg & 80mg) was very similar to that of the control as they all appeared normal but mild changes in the morphology of test groups D and E were observed. These degenerative changes took the form of mild to moderate interstitial congestion and necrosis respectively. These findings are in conformity to previous studies by Eweka *et al.,* (2001) and Tawfik and Al-Badr, (2012) that recorded adverse effects of msg on kidney function and cytoarchitecture. However, there was no study to disprove or to trivialize structural changes in the histology of the liver occasioned by MSG.

Conclusion

The findings of this experimental study after administration of monosodium glutamate (MSG) of different concentrations after a test period of 28days revealed that deleterious changes were severe with increased concentration of MSG treatment. The cytoarchitecture of the kidney tissue shows gross disturbance ranging from mild to severe manifestations. There was also no significant body weight increase in test groups (B, C, D and E) compared to the control group A with significant increase recorded at the fourth week of administration.

Recommendation

Our results suggested that the functions of the kidney could have been adversely affected due to the distortion of the cyto-architecture of the renal cortical structures and cellular necrosis associated with the kidney. It is recommended that;

Further research studies aimed at corroborating these observations be carried out.

Consumers while using MSG as a food additive should be careful of the amount of MSG used considering the fact that it could cause deleterious effects on the kidney at high concentration.

New and refined methods of producing MSG should be looked into so that a healthier MSG can be produced to standard without causing health problem

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AUTHOR'S CONTRIBUTIONS

Idehen, I.C. was involved in development of the research design.
Dic-Ijiewere, O.E was responsible for data collation and manuscript preparation
Airhomwanbor, K.O. was involved in resource and quality control
Ogun, F. E. was responsible for material preparation and execution of the research.
Okparaku, S. O. was involved in resource management and quality control
Ibhawaegbele, S.O. was involved in resource management and quality control
Igwe R. M. N. was involved in the structural logistics.