NEPHROTOXIC EFFECTS OF MONOSODIUMGLUTAMATE IN WISTAR RATS

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Background of study: The use of food additives to enhance the palatability of food has been on for decades. However there are controversies as to the safety of this highly consumed substance. Based on inconsistency in literature and inadequate information available on the effects of MSG on renal function.
Aim: The study was aimed to determine the effects of different doses of MSG on renal function and morphology.
Methods: the rats were randomly assigned into four (4) groups with six (6) rats per group. Group A served as control and were given 2.5ml of water. Group B (Low dose group) were given of 5.3g/kg body weight of MSG. Group C (Moderate dose) treated with 8g/kg body weight of MSG and Group D (High dose) were administered with 16g/kg body weight of MSG. All groups had access to normal rat chow and water for 28 days. Blood and urine samples were collected for the following assays. Creatinine, urea, electrolytes, (sodium, potassium, chloride and bicarbonate) and the kidney collected for histology. Creatinine and urea were analyzed spectrophotometrically, while the electrolytes (sodium, potassium, chloride and bicarbonate) were analyzed using Ion Selective Electrode Analyzer (ISE 400). Urine Albumin was determined using turbidimetric immunoassay method; kidneys were processed and stained with haematoxylin and eosin stain.
Results: There was no significant difference in all the biochemical parameters between control and experimental groups, except for urinary bicarbonate values where rats administered high dose of MSG (18g/kg body weight) had significant increase in urinary bicarbonate when compared with control. There was also no significant increase in the kidney sizes of the various experimental groups when compared with control, but the kidney histology revealed visible renal corpuscle with enlarged glomerulus, interstitial space and tubules with diffused mononuclear cells in the experimental groups.
Conclusion: Data obtained from this study suggests that intake of high concentration of monosodium glutamate results in histological damage to the kidney morphology and also increased excretion of bicarbonate in alkaline urine which can lead to kidney stone formation.
Keywords: Monosodium glutamate, Kidney, Creatinine, bicarbonate, histology, Rats.

INTRODUCTION

The use of food additives to enhance the palatability of food has been on for decades. Monosodium glutamate (MSG), also known as sodium glutamate, is one of the commonly used additives in processed food and Asian cuisine to increase palatability. It is one of the most abundant naturally occurring non-essential amino acids, which has been in used for more than 100 years to season food (Tawfik and Al-Badr, 2012). Trade names of monosodium glutamate include Ac'cent, Aji-No-Moto and Ve-Tsin. The L-glutamate form of MSG confers the same taste of free L-glutamate naturally found in foods (Ikeda, 2002).

The Food and Drug Administration (FDA) has determined MSG as safe for the general population and accordingly stated that an Acceptable Daily Intake (ADI) is not specified (Bellisle 1999, Walker & Lupien 2000). However, recent studies support the hypothesis that MSG consumption may be associated with overweight, metabolic syndrome, or arterial hypertension (He *et al;* 2011, Insawang, *et al;* 2012, He *et.al;* 2008). Also, several studies in animals have shown that MSG is toxic to the various organs such as the liver, brain, thymus, and kidneys (Pavlovic *et al*., 2009). The safety and toxicity of MSG had become controversial in the last few years because of reports of adverse reactions in people who have eaten foods that contain MSG. Many studies had confirmed the adverse reactions of MSG (Meraiyebu *et al*., 2012). The reactions to food containing MSG, known as MSG symptom complex includes; headache, vomiting, nausea, diarrhea, chest pain, sweating, flushing, facial pressure or tightness, irritable bowel syndrome, fluttering heartbeats (heart palpitations), numbness, tingling or burning in the face, neck and other areas, weakness, asthma attacks in asthmatic patients and panic attacks (Obuchi *et al*., 2009). Administration of low doses of MSG (5mg/Kg body weight) resulted in hepatotoxicity in male Wistar rats (Egbuonu *et al*., 2009). MGS tends to increase the number of platelets, bleeding time and clotting time in MSG-treated rats (Meraiyebu *et al*., 2012). High dose of glutamate has been shown to induce significant toxicity in renal culture cells (Leung *e*t *al*., 2008). Increased blood urea nitrogen (BUN) has been associated with kidney disease or failure as well as blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, and shock and bleeding in the digestive tract. Based on inconsistency in literature and inadequate information available on the effects of MSG on renal functions, the aim of this study is to determine the effects of different doses of MSG on some renal parameters.

MATERIALS AND METHODS

Popular brand of monosodium glutamate (Ajinomoto, 99% monosodium glutamate) was purchased in Oba market, Benin City, Edo State.

Preparation of MSG: Varying doses of 5.3g/kg, 8g/kg, and 16g/kg of monosodium glutamate were prepared using distilled water.

Experimental Design: Twenty- four (24) adult male Wistar rats weighing between 150-200g were utilized for this study. The rats were kept in clean plastic cages at temperatures between 18-24°C and under a 12 hr light/dark cycle. The animals received appropriate care in line with the Care and Use of Laboratory Animals guideline of the National Institute of Health. They were allowed access to rat pellet and water *ad libitum* and acclimatized for two weeks before the commencement of the experiment. After acclimatization, the rats were randomly assigned into four (4) groups with six (6) rats per group. Group A served as control and were given 2.5ml of water. Low dose (group B) was given 5.3g/kg body weight of MSG, moderate dose (group C) was treated with 8g/kg body weight of MSG and high dose (group D) was administered with 16g/kg body weight of MSG. Administration was done orally using an oral gastric tube once daily for duration of 8 weeks.

Sample Collection: The rats were put in a metabolic cage over night for urine collection. After which the rats were anaesthetized using chloroform ($CHCl_4$). Blood samples were collected from the rats through the abdominal aorta and cardiac puncture using 5ml syringes and placed in plain tubes. The blood samples were allowed to clot and centrifuged, and the serum was collected to for assay.

Biochemical Assays

Urea estimation: Urea was measured by the Urease- Berthelot (Fawcett and Scott, 1960). Creatinine estimation was measured in both urine and blood by modified Jaffe method (Bartels and Bolumer, 1972). Urine Albumin was determined using turbidimetric immunoassay method by Watanabe *et al*; (1986). For Urinary and blood electrolytes, Ion Selective Electrode was used to measure sodium, potassium, chloride and bicarbonate. Creatinine: albumin ratio was calculated. The kidneys were processed and stained with haematoxylin and eosin (H&E) method by Steven and Alan (1982).

RESULTS

There was no significant difference in serum and urinary sodium, potassium, bicarbonate and chloride concentrations (table1) of the various groups when compared with control except for urinary bicarbonate levels of rats administered high dose of MSG (18g/kg body weight) when compared with control.

Table 1: The mean values of Serum and urinary sodium, potassium, chloride and bicarbonate following administration of Monosodium Glutamate solution at different dosage on rats.

Parameter Electrolytes (mEq/L)	**Control**	**Low Dose (5mg/dl)**	**Moderate Dose (8mg/dl)**	**High Dose (16mg/dl)**
Blood Na^+	145.40 ±.68a	145.00 ±1.58a	146.00 ±1.15a	146.67 ±0.67a
Blood K^+	5.70 ±0.23a	6.82 ±0.35a	5.63 ±0.46a	6.27 ±0.64a
Blood $HCO3^+$	20.80 ±0.58a	21.20 ±0.86a	19.67 ±0.88a	20.67 ±0.88a
Blood Cl-	109.20 ±0.37a	108.00 ±0.86a	108.67 ±0.33a	109.33 ±0.67a
Urine Na^+	22.58 ±2.34a	17.84 ±1.63a	34.00 ±13.01a	32.70 ±17.78a
Urine K^+	190.00 ±2.34a	189.60 ±22.36a	206.67 ±21.86a	246.67 ±35.28a
Urine HCO_3^-	20.60 ±2.34a	18.60 ±1.72a	28.00 ±4.00a	60.67 ±19.33b*
Urine Cl^-	5.28 ±0.95a	5.88 ±0.12a	6.47 ±0.37a	7.00 ±1.06a

*: statistical significant difference from the control.

Results shows no significant differences in serum and urinary concentrations of serum and urinary urea values in the various groups except for the high dose group which showed a significant reduction in serum creatinine concentrations. There was also a significant increase in urinary albumin levels in the high dose group.

Table 2: The mean values of Serum creatinine, urine creatinine and urine albumin, following administration of Monosodium Glutamate solution at different dosage

Parameters	**Control**	**Low Dose (5mg/dl)**	**Moderate Dose (8mg/dl)**	**High Dose (16mg/dl)**
Urea (mg/dl)	50.60 ±1.86a	49.20 ±5.61a	49.33 ±9.56a	50.67 ±9.33a
Serum Creatinine (mg/dl)	0.70 ± 0.04a*	0.74 ±0.05a	0.77 ± 0.03a	0.53 ±0.03*
Urine Creatinine (mg/dl)	5.28 ± 0.95a	5.88 ± 0.12a	6.47 ±0.37a	7.00 ±1.06a
Urine Albumin (mg/dl)	62.64 ± 4.70a	51.28 ±7.11a	78.90 ±30.13a	103.30 ±13.61a*
Creatinine/Albumin	0.09 ± 0.02a	0.12 ±0.02a	0.11 ±0.03a	0.06 ±0.02a

*: statistical significant difference from the control.

Micrograph of the control (Figure 1 and 2) rats reveals normal renal architecture with visible renal corpuscle (long arrow), interstitial space (short arrow) and tubules.

Micrograph of the Kidney

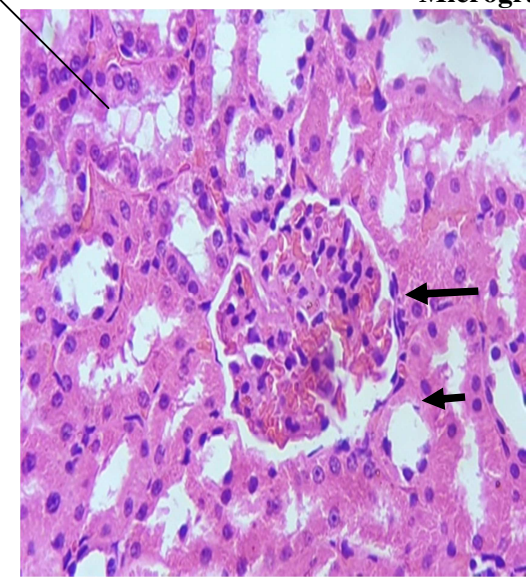


Figure 1: Control distilled water (x400 magnification) Kidney
Kidney: reveals visible renal corpuscle (long arrow), interstitial space (short arrow) and tubules.

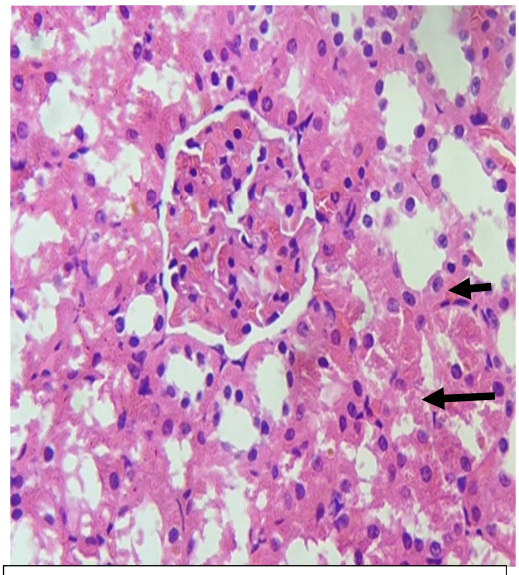


Figure 2: Control distilled water (x400 Magnification)
Kidney**:** reveals visible renal corpuscle (long arrow), interstitial space (short arrow) and tubules.

Micrograph of rats' kidneys (Figure 3) administered low dose of MSG (5.3g) reveals visible atrophied renal corpuscle (long arrow), interstitial, (short arrow) and tubules.

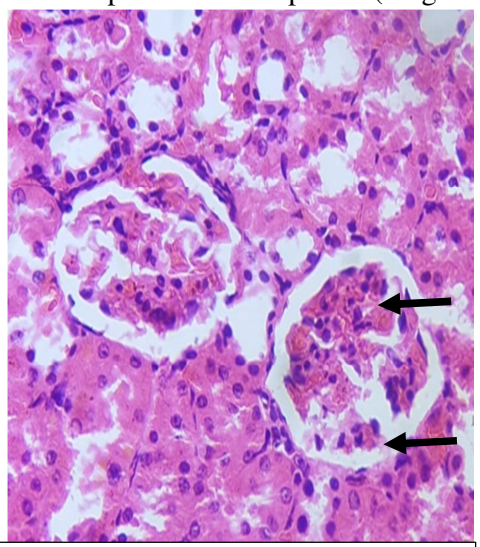
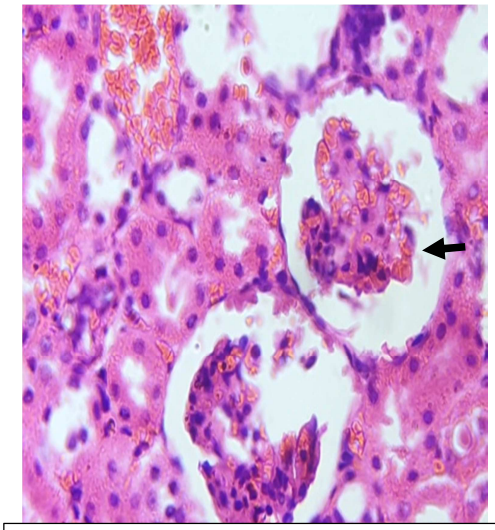


Figure 3: low dose 5.3g (x400 Magnification) Kidney
Kidney: reveals visible renal corpuscle (long arrow), interstitial space (short arrow) and tubules.



Kidney: reveals visible atrophied renal corpuscle (long arrow) and interstitial space (short arrow) and tubules

Micrograph of rat kidneys (Figure 4 and 5) administered moderate dose (8g) of MSG reveals visible renal corpuscle (long arrow), interstitial space and tubules with focal area of mononuclear cells (short arrow).

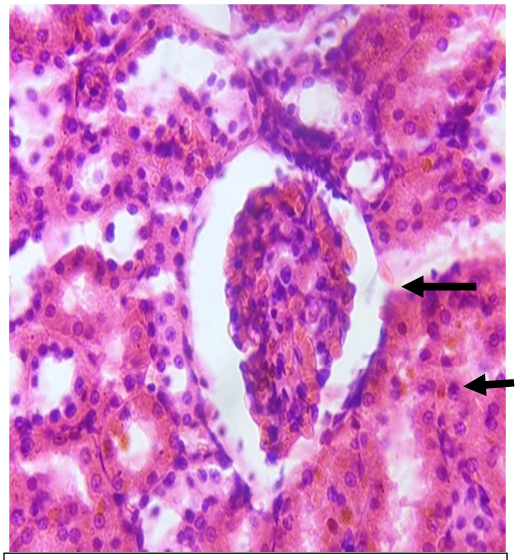


Figure 4: moderate dose 8g (x400 magnification) Kidney
Kidney: reveals visible renal corpuscle (long arrow), interstitial

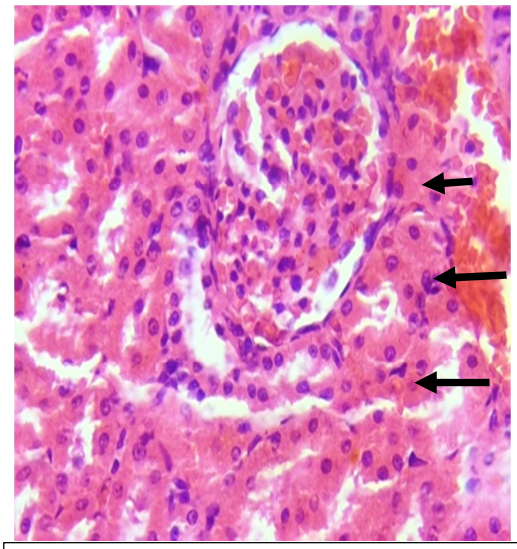


Figure 5: moderate dose 8g (x400 magnification) Kidney
Kidney: reveals visible renal corpuscle with enlarged glomerulus (long arrow) and interstitial space and tubules with diffused mononuclear cells (short arrow).

Micrograph of rats (Figure 6) administered high dose (16g) of MSG showing distorted renal corpuscle (long arrow), interstitial space and tubules with focal area of mononuclear cells and necrosis (short arrow).

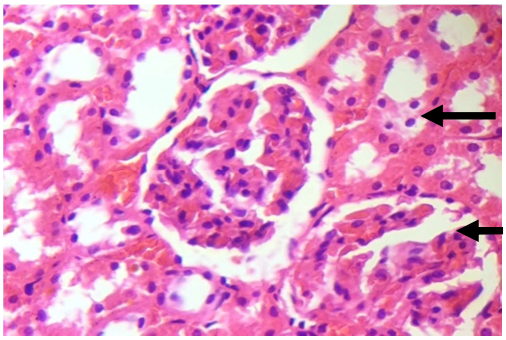


Figure 6: high dose 16g (x400 magnification) Kidney
Kidney: reveals distorted renal corpuscle (long arrow), interstitial space and tubules with focal area of mononuclear cells and necrosis (short arrow).

DISCUSSION

Based on inconsistency in literature and inadequate information available on the effects of MSG on renal functions, we set out to determine the effects of different doses of MSG on some renal parameters and kidney morphology. In this study, we observed no significant differences in blood electrolytes (sodium, potassium, chloride and bicarbonate) levels of control when compared with the experimental groups; however this study revealed a significant increase in urine excretion of bicarbonate in rats administered high dose of MSG when compared with control. Our findings are in agreement with results of De Groot *et al;* (1998), who reported that dietary MSG increases the urinary pH in rats Alkaline urine have been associated with nephrolithiasis.

Alkaline urine may influence the kidney capacity to secrete or reabsorb metabolites that contribute to stone formation, as in the case of calcium phosphate products (Wagner & Mohebbi, 2010). Obstructive nephropathy due to chronic dietary MSG has been reported in adult rats probably due to alkaline urine and decreased levels of stone inhibitors

Plasma creatinine and urea concentrations as well as their urinary values of the various groups administered the various doses of MSG did not show any significant difference when compared with control. This did not agree with the findings of Paul *et.al* (2012), Oritz *et al*, (2006) and Thomas *et al;* (2010), who observed that injection and supplementation of MSG in rat diets induced kidney damage. Tawfik, and Al-Badr, (2012) also observed increased serum creatinine and urea levels in rats administered 0.6, 1.6 mg/g body weight. Refaat *et al;* (2019) reported increased creatinine and urea levels in rats administered 4mg/kg body weight MSG. Although we did not observe any significant difference in serum creatinine and urea levels between control and experimental groups, there was a significant increase in urinary excretion of albumin in the moderate and high doses when compared with control. Moderately increased urinary albumin, also called microalbuminuria, is an early manifestation of diabetic nephropathy, (Gross *et al,* 2005). The significant increase in microalbuminuria measured by the end of this present study reflects changes in glomerular filtration rate, glomerular impairment and failure of the kidney to retain the plasma albumin (Abd-Alaziz *et al*., 2008). It is generally believed that the increased urinary albumin excretion in most renal insults is mostly of glomerular in origin. This may be due to increased intraglomerular pressure, loss of negatively charge in the basement membrane, and increased basement membrane pore size (Marshall, 2004).

While investigating the effect of MSG consumption on kidney morphology, we observed no significant increase in the kidney sizes of the various experimental groups when compared with the control, but the kidney histology revealed visible renal corpuscle with enlarged glomerulus and interstitial space and tubules with diffused mononuclear cells. Endocapillary hypercellularity is a sign of glomerulonephritis (GN) and when diffuse, it's usually due to post-insults to the kidney. Microscopically, aggregates of lymphocytes, plasma cells, monocytes, and fewer neutrophils are randomly scattered or intensely localized throughout an edematous interstitium. Tubular epithelial cells within severely inflamed areas can be degeneratative, necrotic, or both, and profound tubular loss is usually accompanied by eventual replacement fibrosis of the kidney (Melanie & Anthony, 2017), similar results were also reported by Aughey *et al*; (1984) Kjellstom, (1986), Mitsumari *et al*; (1995) and Inkielewicz *et al;* (2003). Bopanna *et al;* (2009) also studied the changes produced by MSG in rats on atherogenic diet on kidney and liver and showed that there was glomerular mesangial proliferation with extensive damage and vacuolation of tubular epithelial cells and infiltrates of inflammatory cells. Eweka (2007) observed distortion of renal cortical structures with some degree of cellular necrosis due to intake of MSG.

CONCLUSION

Data obtained from this study suggests that intake of monosodium glutamate results in histological damages to the kidney morphology. There was also increased excretion of bicarbonate however MSG did not alter urinary and plasma creatinine and urea levels. While we are aware that this study was done in rats, there is need for humans to moderate the intake of MSG.

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