International Journal of Advances in Nephrology Research

3(1): 1-10, 2020; Article no.IJANR.54312

Nephrotoxicity of Monosodium Glutamate (MSG) in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also wrote the manuscript. Author KON managed the analyses of the study and the literature searches. Author ACN wrote the protocol while author JAE performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

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Complete Peer review History: http://www.sdiarticle4.com/review-history/54312

Original Research Article

Received 02 January 2020
Accepted 09 March 2020
Published 25 March 2020

ABSTRACT

Background: Nowadays, monosodium glutamate (MSG) is frequently used as a flavour enhancer, the fact of which makes it one of the most applied food additives in modern nutrition all over the world. But accurate information on the daily intake of specific food additives by individuals is difficult to obtain especially for food additives that are considered to be safe.
Aim: This study sought to investigate the nephrotoxic effect of MSG on Wistar rats.
Methods: Forty Wistar rats were used for this study. Fifteen of the rats were used for acute toxicity test (LD_{50}) and twenty-five for the experiment. Twenty-five (25) Wistar rats were divided into five

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groups of 5 rats each. Animals in groups A, B, C, and D were respectively administered 500 mg/kg, 750 mg/kg, 1000 mg/kg and 1,250 mg/kg b. w. of MSG thoroughly mixed with standard feed for eight weeks. Animals in group E received an equal amount of feeds without MSG added. This group served as the control group. At the end of 8 weeks, animals were fasted overnight and sacrificed under diethyl ether anaesthesia. Renal indices were determined using standard methods.
Results: The LD_{50} was taken to be 500 mg/kg b. w., which is the median of 200 mg/kg b. w. which did not kill any of the animals and 800 mg/kg b. w. that killed all its animals. MSG was observed to increase the concentrations of creatinine, urea, total bilirubin, conjugated bilirubin and unconjugated bilirubin.
Conclusion: The elevation of renal indices by MSG is an indication that it is nephrotoxic.

Keywords: Monosodium glutamate; lethal dose; nephrotoxicity; wistar rats.

1. INTRODUCTION

Nephrotoxicity has been defined as the adverse effect of substances on renal function [1]. These substances include moulds and fungi, cancer therapeutics such as cisplatin, antibiotics such as aminoglycosides, metals such as mercury, arsenic and lead, and drugs of abuse such as cocaine. One indication of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (sCr), or bilirubin concentrations; however, nephro-toxicants can induce kidney damage without changing any established clinical marker of renal function. For example, studies have shown that proximal tubule necrosis in male Sprague Dawley rats exposed to gentamicin can be as high as 75% before any increases in BUN or sCr [2].

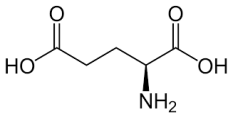

Fig. 1. Structure of glutamate [4]

Monosodium glutamate (MSG) is a sodium salt of glutamic acid. It is usually a white powder. Water ionizes it into free sodium ions and glutamic acid, which is an organic compound consisting of five carbon atoms. It has a carboxylic (-COOH) group and an amino ($-NH_2$) group attached to an "alpha" carbon atom (a carbon atom joined directly to the – COOH group). It is an alpha-amino acid. The molecular formula of MSG is $C_3H_8NNaO_4$ and its molecular mass is 169.11 $gmol^{-1}$. MSG has the same basic structure of amino acids, with an amine group ($-NH_2$) and carboxylate ion instead of the carboxylic group ($-COO^-$). MSG has the almost same structure with glutamate. The difference is that one hydrogen atom at the carboxylic chain has been replaced with a sodium atom, hence, the name monosodium glutamate [3,4].

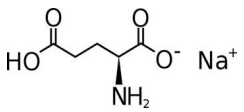

Fig. 2. Structure of monosodium glutamate [4]

Monosodium glutamate (Fig. 2) has a distinctive taste that falls outside the region of the four classic tastes: sweet, sour, salty, and bitter. This taste is called “Umami,” also referred to as “Xien Wei" in Chinese or "savoury, "broth-like" or "meaty taste" in English [4]. Owing to this unique taste, many producers of food use MSG to enhance the flavour of their product [5]. Recently, Chaudhari et al. [6] identified a specific glutamate taste receptor on the tongue. Three umami substances (glutamate, 5-inosinate, and 5-guanylate) were found by Japanese scientists, but umami has not been recognized in Europe and America for a long time. In the late 1900s, umami was internationally recognized as the fifth basic taste based on psychophysical, electrophysiological, and biochemical studies. Three umami receptors (T1R1 + T1R3, mGluR4, and mGluR1) were identified. There is a synergism between glutamate and the 5-nucleotides. Among the above receptors, only T1R1 + T1R3 receptor exhibits the synergism [7]. Since glutamate and 5-inosinate are contained in various foods, umami taste is induced by the synergism in daily eating [4,7].

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 [4,8].

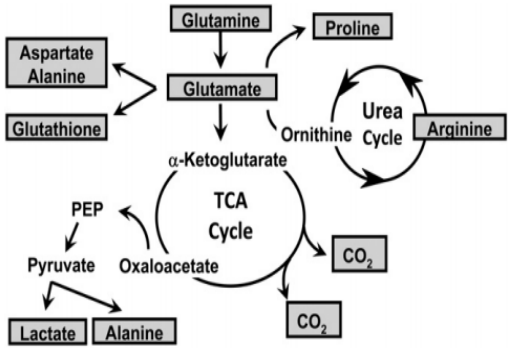

Fig. 3. Metabolic fates of dietary glutamate in the intestine [4]

MSG could lead to headache, vomiting, diarrhoea, irritable bowel syndrome, asthma attacks in asthmatic patients and panic attacks [4]. Obuchi et al. [9] studied the effect of garlic extracts on MSG induced fibroid in Wistar rats and reported that MSG alone increased total protein, cholesterol and estradiol (estrogen), which in turn, induced fibroid in the rats. However, treatment with garlic extracts near-completely abrogated/mitigated any effects that have been induced by MSG alone [4]. Egbuonu et al. [10] reported a study aimed at investigating the potentials of low concentration administration of monosodium glutamate in inducing hepatotoxicity in male albino rats. In that study, it was observed that treating rats with monosodium glutamate at a low concentration (5 mg/kg of body weight) could be hepatotoxic without significant cholestasis or pathologies of the bone [4]. Onyema et al. [11] reported that MSG at a dose of 0.6 mg/g body weight induced the oxidative stress and hepatotoxicity in rats and vitamin E ameliorated MSG-induced oxidative stress and hepatotoxicity. Meraiyebu et al. [12] reported that MSG increased the number of platelets, bleeding time and clotting time in MSG-treated rats. Onyema et al. [13] tested the hypothesis that alteration in glucose metabolism following MSG administration might be a contributor to the changes in the markers of oxidative stress observed in the animals. The pattern of induction of oxidative stress and alteration of glucose metabolic enzymes in the animals was an indication that oxidative stress induced by MSG in the renal tissues of rats might be contributed by increased tissue glucose concentration resulting from enhanced renal gluconeogenesis [13]. Nwajei et al. [14] reported that four selected food seasonings (labelled as IS, KC, SMC and BS) commonly consumed in Nigeria adversely perturbed some sex hormones: testosterone, estrogen and progesterone of Wistar albino rats due to the presence of MSG in these seasonings. Kolawole [15] investigated the effect of orally administered MSG on food consumption, body weight and some biochemical and haematological parameters in adult Wistar rats and reported that MSG at the doses or 5 – 15 mg/kg body weight was not hazardous to health. This study sought to evaluate the effect of monosodium glutamate in renal indices of Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection of Monosodium Glutamate (MSG)

The MSG (3 g/sachet containing 99% MSG) was obtained from a Grocery Store at New Market, Aba in Abia State, Nigeria.

2.2 Collection of Animals

Forty (40) male Wistar rats with bodyweight between 160 and 200 g were obtained from the animal house of the Department of Pharmacology and Therapeutics, College of Medicine and Health Science, Abia State University, Uturu, Nigeria. They were acclimatized for seven days before the study. All the animals were handled by the standard guidelines for care and use of laboratory animals. The animals had access to standard animal feed purchased from a local commercial supplier and water *ad libitum* and housed under a standard condition of temperature (25±2ºC) under 12 hours light-darkness cycles. Fifteen (15) of the rats were used for acute toxicity test and twenty-five (25) for the experiment.

2.3 Acute Toxicity Test (LD_{50}) Determination

The acute toxicity test (LD_{50}) was determined using a modified version of the method proposed by Lorke [16] which involves the use of the minimal number of experimental animals. This method of acute toxicity determination makes the following assumptions.

I. Substances more toxic than 1mg/kg body weight are so highly toxic that it is unnecessary to calculate the LD_{50}.
II. LD_{50} values greater than 5000 mg/kg is of no practical interest.

III. An approximate figure for the LD_{50} is usually adequate to estimate the risk of acute intoxication.

The LD_{50} is taken as the median concentration that killed 50% of the test animals. The median lethal dose was estimated as the geometric mean of the last dose at which none of the animals died and the highest concentration at which all the animals died. The 15 animals used in the determination of LD_{50} were divided into five groups of 3 each. Groups A, B, C and D were administered 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg of MSG respectively through the intraperitoneal route of drug administration while group E was similarly treated but with saline solution. This group served as the control group. The animals were constantly observed for 24 hours for signs of toxicity and death.

2.4 Experimental Design

A total of twenty-five (25) male Wistar rats were divided into five groups of 5 rats each. Animals in groups A, B, C, and D were respectively administered 500 mg/kg, 750 mg/kg, 1000 mg/kg and 1,250 mg/kg of MSG thoroughly mixed with standard feed for 8 weeks. Animals in group E received an equal amount of feeds but without MSG. This group served as the control group. At the end of 8 weeks, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture.

2.5 Determination of Renal Indices

Creatinine concentration was determined using the Jaffe reaction described by Toora and Rejagopal [17]. Urea concentration was determined using a Randox Commercial Kit based on the methods of Fesus et al. [18]. Total bilirubin concentration was determined by the diazo method described by Royden and Alfred [19]. Conjugated bilirubin concentration was determined by the method of Compernolle [20]. Unconjugated bilirubin was determined by subtracting conjugated bilirubin from total bilirubin.

2.6 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS), version 20.0. Results were presented as Mean ± Standard Error of the mean (SEM). 2–tailed t-test was used for comparison of the means. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

3.1 Acute Toxicity Test

One of the animals in Group D (administered 800 mg/kg body weight) died within the first 30 minutes of administration. After 12 hours of observation, another one died in Group D. The remaining one in group D and one in group C died overnight. The LD_{50} was then taken to be 500 mg/kg, which is the median of 200 mg/kg which did not kill any of the animals and 800 mg/kg that killed all its animals.

3.2 Systemic Effect of MSG

Two weeks into the study, most of the animals in the experimental group became hyperactive. Four weeks later, one of the animals in the group fed with 1250 mg/kg of MSG developed bulging of eyeballs (exophthalmos) and had several bouts of seizures before its demise six days later.

3.3 Effect of MSG on Renal Indices

The result of the effect of MSG on renal indices is presented in Figs. 4-8.

4. DISCUSSION

Noxious substances abound in the environment. We come in contact with them every day, either directly or indirectly. In either way, they have an effect on our health. Some examples of these substances are carbon monoxide (CO), alcohol, tobacco, and additives such as Aspartame, Sulfites, Nitrates/nitrites and MSG. Consumption of MSG worldwide with its attendant toxic effect remains a concern to medical and food scientists. This study sought to evaluate the effect of monosodium glutamate in renal indices of Wistar rats.

Studies on the tissue biomarker alterations might reflect the metabolic abnormalities and cellular injuries in some organs. The liver and kidney have an extremely important function in detoxification and excretion of metabolic wastes and xenobiotics [21]. Exposure to toxic chemicals causes alterations in some tissue enzyme activities. The kidneys control the excretion of

urea, creatinine, and reabsorption of electrolytes into the blood. Defeat in activities of the kidney will result in accumulation of electrolytes, urea, and creatinine in the biological fluid [22]. The results of renal indices of animals sequel to treatment with MSG is presented in Figs. 4-8.

Yohei et al. [23], reported that the relationship between high renal resistive index (RI) and cardiovascular and renal outcomes is significant and persisted after multivariate Cox regression analysis, including traditional risk factors. The serum creatinine concentration is widely interpreted as a measure of the glomerular filtration rate (GFR) and it is used as an index of renal function in clinical practice [24]. Glomerular filtration of creatinine, however, is only one of the variables that determine its concentration in serum. Alterations in renal handling and metabolism of creatinine and methodological interferences in its measurement may have a profound impact on the serum concentration of creatinine metabolism and are constant among individuals and over time, with the creatinine production rate being equal to the renal excretion rate. In the theoretical situation where both criteria are satisfied, the serum creatinine is inversely proportional to the GFR, so that each halving of the GFR results in a doubling of the serum creatinine concentration [25]. Secretion of creatinine was observed even in early studies of

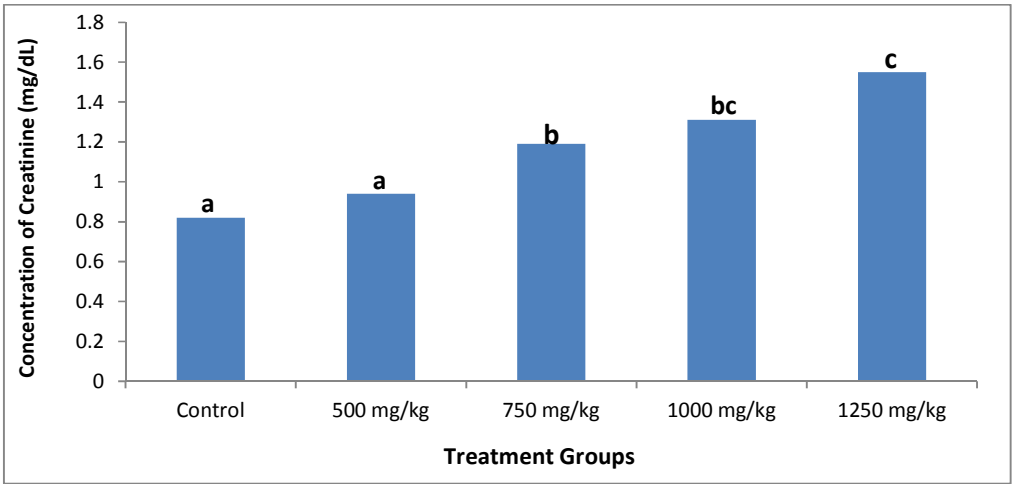

Fig. 4. Effect of MSG on the concentration of creatinine after 8 weeks of treatment

Bars represent mean±standard deviation with n = 5. Bars with different letters are significantly different at P<0.05

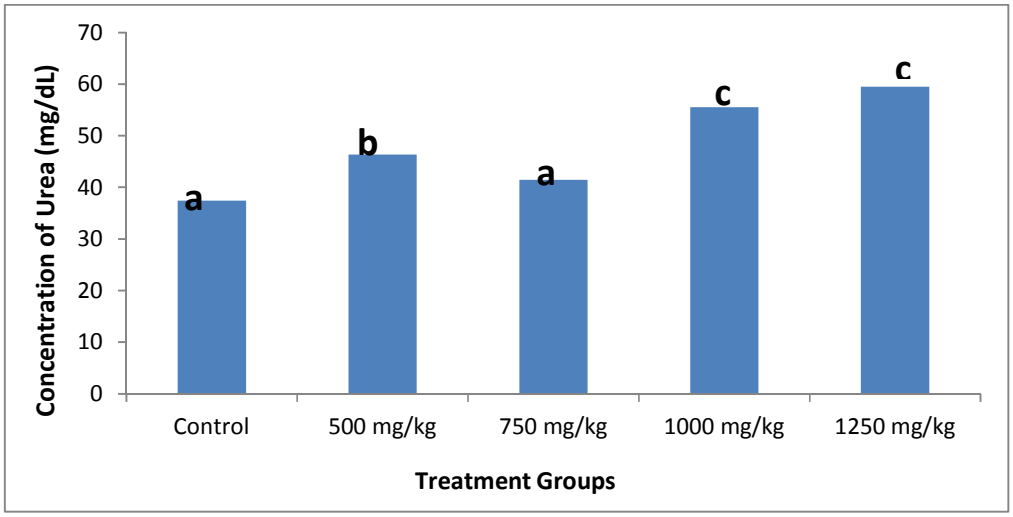

Fig. 5. Effect of MSG on the concentration of urea after 8 weeks of treatment

Bars represent mean±standard deviation with n = 5. Bars with different letters are significantly different at P<0.05

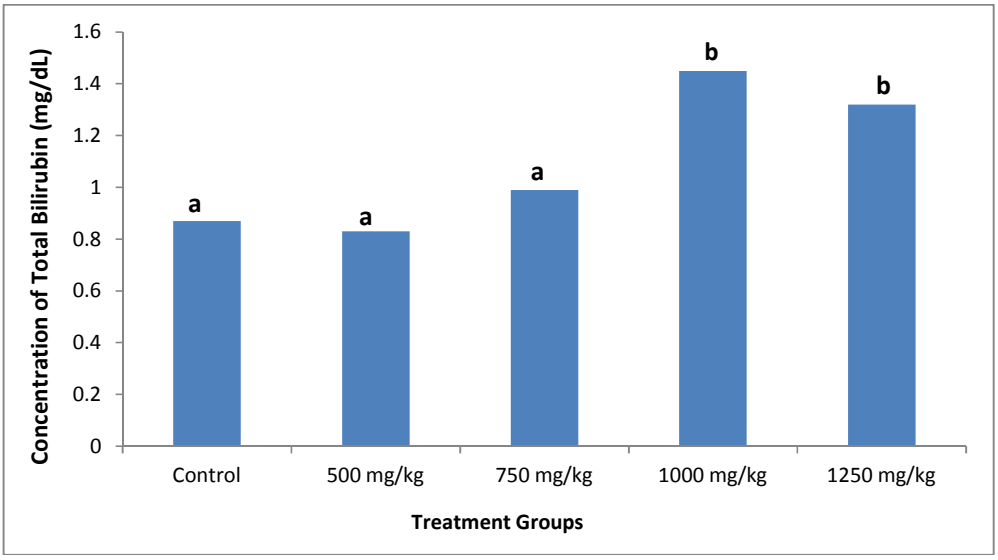

Fig. 6. Effect of MSG on the concentration of total bilirubin after 8 weeks of treatment
Bars represent mean±standard deviation with n = 5. Bars with different letters are significantly different at P<0.05

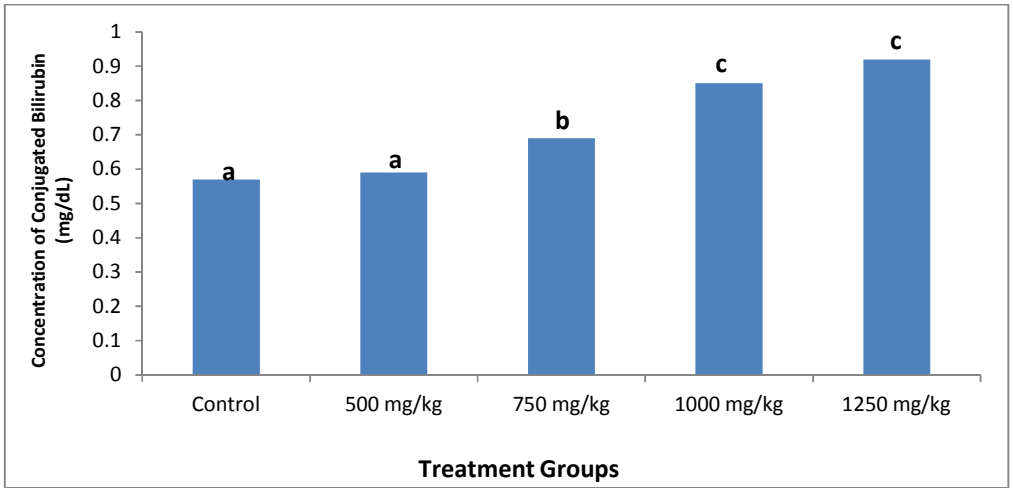

Fig. 7. Effect of MSG on the concentration of conjugated bilirubin after 8 weeks of treatment
Bars represent mean±standard deviation with n = 5. Bars with different letters are significantly different at P<0.05

the clearance of exogenously administered creatinine [25]. In 2003, Mandell et al. [24], reported that the exogenous creatinine clearance decreased as the concentration of creatinine in the blood was acutely increased10-fold by creatinine infusion. This decrease was thought to be due to saturation of the tubular secretory mechanism because the inulin clearance was not affected by this exogenous increase of the creatinine concentration in the blood. Creatinine reabsorption during low rates of urine flow is thought to result from its passive back-diffusion from the lumen to the blood. Thus, when the urine flow rate is very low, passive reabsorption of creatinine might result in lower creatinine clearance and a higher concentration of serum creatinine than what one would expect solely based on the GFR [24,26]. Dietary protein deficiency leads to negative nitrogen balance and loss of muscle mass, thereby decreasing creatinine production. Less severe alterations in the diet, however, also may have important effects on the size of the creatine pool and creatinine excretion, which are independent of nitrogen balance and muscle mass.

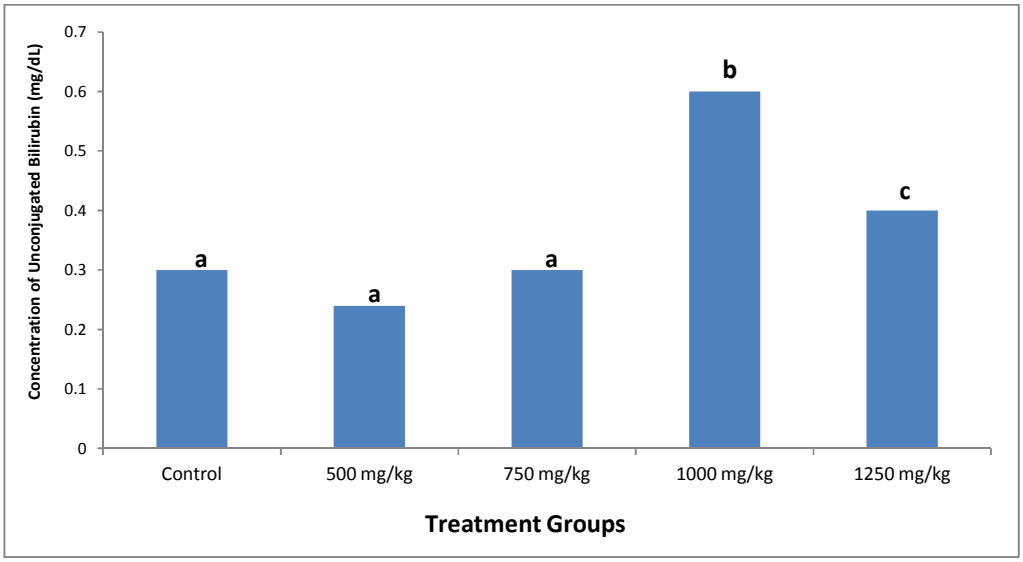

Fig. 8. Effect of MSG on the concentration of unconjugated bilirubin after 8 weeks of treatment
Bars represent mean±standard deviation with n = 5. Bars with different letters are significantly different at P<0.05

In this study, a dose-dependent increase was observed in the serum creatinine concentration of experimental animals when compared with that of the control animals (Fig. 4). This increase was significant at the doses of 750, 1000 and 1500 mg/kg body weight. The significant increase in creatinine content at these doses may be attributed to compromise of the renal functional capacity. MSG might have altered creatinine metabolism in favour of increase anabolism, decrease catabolism and decrease clearance [22]. This corresponds to the findings of Abass and El-Haleem [27]. The negative effects of monosodium glutamate were first observed in newborn mice by Lucas D R and Newhouse J P, 1957 who noted that monosodium glutamate-induced elevation of glutamate level in the brain of mice that end in the destruction of the neurons in the inner layers of the retina. Later, in 1969, John Olney discovered the phenomenon was not restricted to the retina, but occurred throughout the brain, and coined the term excitotoxicity [22,27].

Serum urea and creatinine levels are an indication of kidney function both in man and in rodents. In this study, an increase was observed in the serum urea concentration in experimental animals when compared with those of the control animals (Fig. 5). This increase was only significant at the dosage of 1000 mg/kg and 1500 mg/kg body weight respectively. The significant increase in urea content at these doses may be attributed to compromise of the renal functional capacity. MSG administration at these doses might have perturbed urea metabolism in favour of increase anabolism, decrease catabolism and decrease clearance.

Bilirubin is the breakdown product of heme moiety of haemoglobin; other hemoproteins include cytochromes, catalase, peroxidase, tryptophan pyrroles and a small pool of free heme. Increase in concentration of direct reacting bilirubin in blood causes hyper-bilirubinaemia, which is toxic under certain conditions inducing jaundice, hyperbilirubinemia-induced auditory dysfunction and neurotoxicity resulting in brain damage [28]. On the other hand, mild unconjugated hyperbilirubinaemia behaves as a mild antioxidant and might offer protection against cardiovascular diseases and tumour development [29]. Recent research survey has reported that low concentration of direct reacting bilirubin induces stroke in body and sometimes causes cardiac problems too. Serum bilirubin levels are often enhanced under a variety of clinical conditions. In the circulation of blood, bilirubin is bound to serum albumin, which prevents its potential toxicity thought to be caused by free bilirubin [30]. Despite its high-affinity of binding to albumin, bilirubin is rapidly and selectively taken up by the liver, biotrans-formed upon conjugation with glucuronate, and secreted into bile [31]. Thus bilirubin is converted into bilirubin glucuronic acid in the liver and excreted along with bile.

Free bilirubin is the breakdown product of haemoglobin (Hb) of aged erythrocytes in the reticuloendothelial cells of the spleen. This free bilirubin is not bound to albumin and its toxic effect is believed to occur even at a concentration of 0.005 mg/dL. So far, no reliable method has been developed for measuring free bilirubin content in plasma (or for measuring the free binding capacity of albumin for free bilirubin). The free bilirubin bound to albumin is called unconjugated bilirubin. The splitting of heme ring at different positions (α, β, γ or δ) leads to the formation of its various isomers which cannot form hydrogen bonds, and are therefore more readily water-soluble and get excreted through the urine [32].

The free or unconjugated bilirubin bound by albumin is carried to the liver, where it is conjugated with glucuronic acid by the enzyme glucuronyltransferase. The enzyme, glucuronyltransferase transforms the albumin-bound bilirubin to monoglucuronide or diglucuronide conjugated bilirubin urine [32].

Total bilirubin has been reported to be a potent physiologic antioxidant that may provide important protection against atherosclerosis, coronary artery, and inflammation [33], total serum bilirubin level concentrations are directly proportional to the protective factor high-density lipoprotein-cholesterol [34]. Increase in total bilirubin, conjugated and unconjugated bilirubin level in the experimental animals, when compared with those of the control group, might have resulted from kidney damage following MSG administration.

5. CONCLUSION

The significant increase observed in all the renal indices of animals treated with MSG when compared with those in the control group showed that MSG is nephrotoxic.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic Committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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