EFFECT OF MONOSODIUM GLUTAMATE ON THE LIVER AND KIDNEY FUNCTION OF ADULT ALBINO RATS AND THE PROTECTIVE POTENTIALS OF VITAMIN E

Ezeokeke C. T.[1] and Ezekwe O.C.[2]

Department of Nutrition and Dietetics, Faculty of Health Sciences, Imo State University, Owerri. P.M.B 2000.
Author of correspondence: Ezekwe Odirirchukwu Chika. Email: odyfidel@gmail.com

ABSTRACT

Background of the study: Monosodium Glutamate (MSG) is one of the world's most extensively used food additives which is ingested as part of commercially processed foods. MSG produces a flavor that cannot be provided by other foods. It elicits a taste described by Japanese as umami, which is translated to "savory".
Materials and methods: A total 24 male adult albino rats weighing (80-120 g) were used in the study. They were randomly assigned to 4 groups, and six rats per groups and three replicates of two rats per replicate. Group 1 served as the control group and the groups 2, 3, and 4 served as the test groups. MSG was administered to rats at dose of 0.6 mg/g body weight for 30 days.
Results: Body weight and relative liver and kidney weights of the rat significantly increased. Regarding to liver functions, the activities of alanine transferase (ALT) and aspartate transferase (AST) significantly ($P<0.05$) increased in the serum on MSG administration, meanwhile serum total protein, albumin and serum total bilirubin significantly ($P<0.05$) decreased. Serum urea and creatinine were significantly ($P<0.05$) increased. In the histology, the kidney of the rats treated with MSG had early degeneration changes like glomerular shrinkage, affected urinary tubules and connective tissues while the liver had depleted cytoplasm, eroded endothelial layer and degenerated nuclei. Vitamin E co-administered with MSG, significantly restored the body weight and the relative liver and kidney functions to slightly controlled levels.
Conclusion: The results showed that MSG at the dose of 0.6 mg/g body weight may cause an adverse effect on the hepatic and renal function which may be due to oxidative stress induced by MSG on the liver and kidney tissue. Supplementation of vitamin E was capable of ameliorating MSG-induced oxidative stress on hepatic and renal functions.
Keywords: Monosodium Glutamate; Hepatic and Renal Stress; Vitamin E; Glomerular; Cytoplasm.

INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of the amino acid glutamic acid. Glutamic acid or glutamate is one of the most common amino acids found in nature. It is the main component of many proteins and peptides, and is present in most tissue. It is made commercially by the fermentation of molasses, but exists in many products made from fermented proteins, such as soy sauce and hydrolyzed vegetable protein. Glutamate is also produced in the body and plays an essential role in human metabolism [1]. The consumption is higher in the oriental countries than in the western countries. This is due to traditional oriental cooking which uses a lot of condiments to supplement; enhance or round off cooking such as the flavors of many savory based processed foods, for example soy sauce [2]. Glutamate is the excitatory neurotransmitter in the mammalian central nervous system (CNS) playing an important role in both physiological and pathological processes [3]. It has been reported that MSG has neurotoxic effects resulting in brain cell damage, retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, parkinson's disease, huntington's disease, and amyotropic lateral sclerosis [4]

The potential link between MSG and obesity includes the MSG effect on energy balance by increasing palatability of food and by disrupting the hypothalamic signaling cascade of leptin action [5]. This is to say, the more palatable the food is, the more you eat and when this happens on a long period of time, obesity sets in. MSG could produce symptoms like numbness, weakness, flushing, sweating, dizziness and headache. Syncope, and facial pressure may be experienced later [6]. They are collectively known as Chinese Restaurant Syndrome. 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 albino rats. It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology [7]. However, the U.S food and Drug administration (FDA) designated MSG as "Generally Recognized as Safe" (GRAS) [8] in any given quality.

Liver is the largest gland in the mammalian body. The hepatocytes have metabolic functions that handle the essential processes such as the take up glucose, minerals, and vitamins from the portal and systemic blood and store them. In addition, hepatocytes can produce many important substances needed by the body; such as cholesterol and glucose, the liver helps maintain body homeostasis [5, 6]. The liver also stores fat-soluble vitamins (vitamin A, D, E, and K), vitamin B_{12} and minerals such as copper and iron [9].The liver removes harmful substances (such as ammonia and toxins) from the blood and then breaks them down or transforms them into less harmful compounds [9]. In addition, the liver metabolizes most hormones and ingested drugs to either more or less active products. Several enzymes have been determined to detect the hepatic status such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [9].

Kidney is a paired organ located in the posterior abdominal wall. The major functions include the removal of toxic metabolites and waste products from the blood and the regulation of the amount of fluid and electrolytes balance in the body. To test functions of the kidney routine urinalysis is used to measure serum urea, creatinine, sodium, potassium and serum bicarbonate (10, 11].

Vitamin E (alpha-tocopherol) is the primary membrane bound, lipid-soluble, chain-breaking antioxidant that protects cell membranes against lipid peroxidation [12]. Vitamin E pre-treatment has been reported to be beneficial in preventing, formaldehyde-induced tissue damage in rats [13].

MATERIALS AND METHODS

SAMPLE PROCUREMENT

A total of 24 adult male albino rats weighing (80-120g) were obtained from Animal Care Center, Anatomy Laboratory, Imo State University. The rats were allowed to acclimatize for two weeks and monitored daily during the 6 weeks of the study. Their body weights were measured daily. The animals were housed in plastic cages under standard hygienic condition and had free access to commercial feed of 200g measured with beam balance and water. Monosodium Glutamate (Ajinomoto) was purchased from Anatomy Laboratory Imo State University, and pure vitamin E (25g) was purchased at PAC BESH SCIENTIFIC CO. (NIG). LTD. Number 2, Uratta street, Owerri, Imo state.

EXPERIMENTAL DESIGN

The rats were distributed to treatment blocks using completely randomized design. Each treatment contained six rats and each treatment had three replicates of two rats per replicate.

TREATMENTS

Treatment 1 - Control (200g Normal diet)
Treatment 2 – Normal diet + 0.6mg/g body weight plus addition of MSG [14].
Treatment 3- Normal diet + 0.2mg/g body weight plus addition of vitamin E [14].
Treatment 4 – Normal diet + 0.6mg/g body weight plus addition of MSG and 0.2mg/g body weight vitamin E [14].

Sample Collection: The animals were fasted for 24 hours after 30 days, and then administered with chloroform as an anesthesia.
Body Weight: The body weights of the rats weighing 80-120g were grouped according to four treatments 1-4. During the measurement, each animal from each group is placed on the weighing balance to obtain its weight. MSG and vitamin E administration were given according to the mean weight of the rats per group.
Organ Weight: The liver and the kidney were removed, washed in ice-cold 1.5% KCl to remove blood and extraneous substances, dried in a filter paper and weighed, then kept in the fridge for bioassay.
Blood Collection: About 4-5 ml of whole blood was collected by cardiac puncture into a plain glass tube, allowed to clot, retracted and centrifuged at 4000 rpm for 5 minutes. The serum was separated into a plain container and stored at -80^{O}C before analyses.
Specimen Storage and Handling during Testing: Samples were received frozen and stored at -80 ^{O}C until testing is performed. Upon completion of analysis, specimens were stored at -80^{O}C.

Determination of Bilirubin: Bilirubin was determined with the method of Malloy and Evelyn [15].
Determination of Alanine transferase (ALT) and Aspartate transferase (AST): The activities of ALT and AST were determined by the method of Reitman and Frankel [16]. All reagents for the assay were obtained from Randox (UK).
Determination of albumin: Albumin was determined by the modified method of Bartholomew and Delany [17].

Determination of Total Protein: Protein was determined with Biuret method.

Determination of Urea: Urea was determined using Diacetylmonoxime method [18].

Determination of Creatinine: Creatinine was determined with the method of (Jaffes reaction) [19].

HISTOLOGICAL METHOD

Tissue Processing: Following fixation, the gross anatomy was noted and sections were taken and histologically processed by manual tissue processing method. The tissue processing involves the various stages between fixation and cutting of section [20, 21].

Staining techniques: Paraffin section after they have been cut was attached to the slides and routine hematoxylin and eosin (H&E) staining method was used [21].

Microscopy and Photomicrophy: The sections were examined using Olympus binocular microscope with an inbuilt lifting system. The sections were photomicrographed using a digital camera on Olympus photomicroscope.

Statistical Analysis

All values were expressed as mean ± SEM. The statistical analysis was carried out using ANOVA i.e analysis of variance and separation of means using statistical package for social sciences version (SPSS) 20 at $P<0.05$.

RESULTS

ORGAN WEIGHTS

Table 1 shows the effect of MSG, vitamin E and MSG + vitamin E on the liver and kidney weights of the male albino rats. There was a significant difference ($P<0.05$) in the liver and kidney weights of the rats treated with MSG groups of the liver and the kidney at 7.42g and 0.82g respectively when compared with the control group at 4.97g and 0.58. MSG + vitamin E groups at 6.15g and 0.63g of both the liver and the kidney respectively showed a significance difference when compared with the MSG groups at 7.42g and 0.82 which proved the effectiveness of the vitamin E on MSG. The changes in relative liver and kidney weights have been accompanied with changes in functional aspects. There was no significant difference between the vitamin E group of the liver and kidney at 5.01g and 0.51g respectively when compared with the control group at 4.97g and 0.58g.

Table 1: the effect of MSG, vitamin E and MSG + vitamin E on the liver weight

Parameters	Control	MSG	Vitamin E	MSG+ E	Vitamin	±SEM
Liver (g)	4.97^{c}	7.42^{a}	5.01^{c}	6.15^{b}		0.26
Kidney(g)	0.58^{b}	0.82^{a}	0.51^{b}	0.63^{b}		0.05

Note: *Mean values were expressed in mean ± SEM. Mean values along a row with different superscript differs significantly ($P<0.05$). SEM = standard error of mean.*

BLOOD ANALYSIS

Table 2 shows the effects of MSG, vitamin E and MSG + vitamin E on the total protein, albumin, bilirubin, globulin, AST and ALT of the liver function test of male albino rats. The determined mean value of total protein in serum of control rats was 5.40g/dl. The reduction in serum total protein of MSG group at 4.50g/dl dosed rats was not significance ($P>0.05$) level (Table 2). Total protein of the rats treated with vitamin E showed the highest mean ($P>0.05$) value compared with others. Albumin concentration decreased but not significant in the serum MSG dosed rats. The synthetic function of the liver was altered by MSG, so albumin levels decreased. Albumin concentration increased ($P>0.05$) in the serum of MSG administered by additional vitamin E dosed rats. Table (2) shows the activities of serum alanine aminotransferase (ALT) enzyme that were measured in the serum samples. The group treated with MSG + vitamin E, neither significant increase nor reduction was observed among the treatment in ALT and AST levels of the rate. Bilirubin showed no significant difference between the MSG-treated rats, the control, and MSG + vitamin E groups, but rats on vitamin E showed a significant reduction ($P<0.05$) in ALT when compared to others in the other treatments. Globulin was reduced significantly ($P<0.05$) of rats on the MSG when compared with the control and those on diet treated with vitamin E.

Table 2: the effect of the total protein, albumin, bilirubin, AST and ALT of the liver

Parameters	Control	MSG	Vitamin E	MSG +Vitamin E	±SEM
Total protein (g/dl)	5.40	4.50	5.90	5.43	0.20
Albumin (g/dl)	3.40	3.10	3.57	3.50	0.20
Globulin (g/dl)	2.00[ab]	1.40[b]	2.33[a]	1.93[ab]	0.19
Total bilirubin(µmol/l)	9.80	11.43	9.47	9.53	0.95
Conjugated bilirubin(µmol/l)	3.60	3.27	3.17	3.43	0.35
Unconjugated bilirubin(µmol/l)	6.20	8.17	6.30	6.13	0.83
AST (u/l)	47.67	66.67	49.33	54.67	12.79
ALT (u/l)	36.33[a]	43.67[a]	21.33[b]	36.67[a]	2.62

Note: *Mean values were expressed in mean ± SEM. Mean values along a row with different superscript differs significantly (P<0.05). SEM= Standard error of mean.*

Table 3. Shows the effect of MSG, vitamin E and MSG+ vitamin E on kidney function test

Blood serum of samples of the rats treated with MSG (8.47mmol/L) contained low mean value urea as compared with others.

Concentrations of creatinine of the MSG treated rats were found to have a higher mean value than that of the control group. The difference between means were not significant (P<0.05).

Table 3: the effect of MSG, vitamin E and MSG+ vitamin E on kidney function test

Parameters	Control	MSG	Vitamin E	MSG+ vitamin E	±SEM
Urea	10.23	8.47	10.33	9.17	1.65
Creatinine	45.97	58.00	43.17	43.63	6.91

Note: *Mean values were expressed in mean ± SEM. Mean values along a row with same superscript do not differ significantly (P<0.05). SEM = Standard error of mean.*

BODY WEIGHT

Table 4 shows the effect of MSG, vitamin E and MSG + vitamin E on the body weight of albino rats.

The body weight of the rats across the treated groups differed nominally when compared among the groups. The initial and the final mean weights of the four groups investigated lied between 90.00g and 163.33g.

Table 4: the effect of MSG, vitamin E and MSG + vitamin E on the body weight of the rats.

Parameter	Control	MSG	Vitamin E	MSG+ vitamin E	±SEM
Initial weight(g)					
Final weight(g)	90.00	96.67	100.00	103.33	12.02
	116.67	163.33	133.33	140.00	12.91

Note: Mean values with different superscript along a row differs significantly (p<0.05), values were expressed in means ± SEM (Standard error of mean)

HISTOLOGY
KIDNEY

Plate 1 Control: The photomicrograph of the section of the renal cortex showing an enlarged glomeruli(G). the endothelium are intact, the urinary space appeared normal. The urinary capsule of its linings(UCL) are intact as distinct. Juxtaglomeruli apparatus (JGA) were also intact. The urinary tubule (UT) shows with contracted lumen but the lining are normal. The connective tissues are intact.

Plate 2 MSG: Photomicrograph of the section of the renal cortex slowly the glomeruli that are either degenerating or completely depleted. This created a wider urinary space completely different from the normal section. The depletion also affected the urinary tubules as connective tissues. The nuclei appear tiny and numerous.

Plate 3 Vitamin E: Photomicrograph of the section of the renal cortex showing the glomeruli with liquid endothelium. The juxtaglomeruler apparatus were partly intact. There are gross cellular depletion of the cells of the organ. The tubules appears to retain some fluid in their lumen. The nuclei of the organ also appeared tiny than normal.

Plate 4 MSG + Vitamin E: The photomicrograph of the section of the renal cortex showing the glomeruli that are either depleting in nature or completely depleted. Wider urinary space is created in this section than any other section. The depletion also affected the cells forming the juxtaglomeruler apparatus and the urinary tubules. The nuclei were also tiny

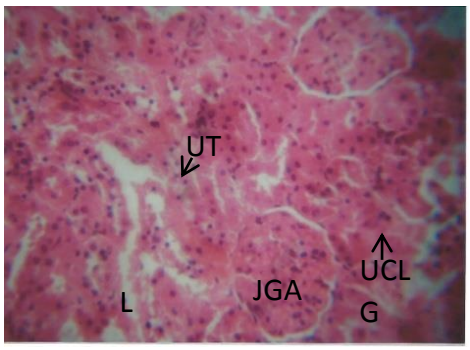
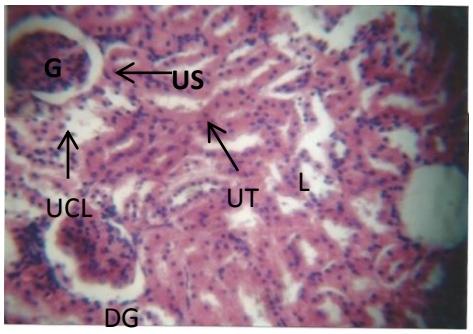


PLATE 3: cross section of the control rat(group 1) kidney showing normal glomeruli (G). Intact endothelium (E), urinary capsule linings (UCL) and Juxtaglomerular (JGA) plus normal lumen (L). (Photomicograph x 400).



DDG

PLATE 4: A cross section of the MSG (group 2) rat of the renal cortex showing degenerating or compete depleted glomeruli (DDG), urinary tubules (UT) and tiny nuclei (N). (Photomicrograph x400)

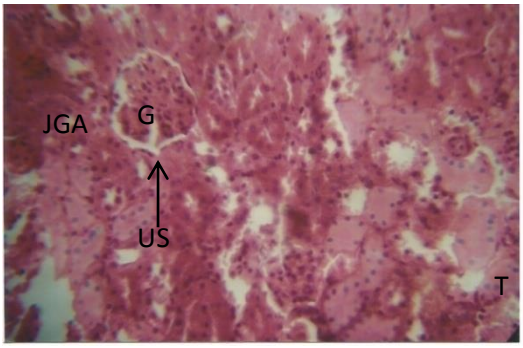


PLATE 5: a cross section of the vitamin E (group 3) rat of the renal cortex showing glomeruli (G) with liquid endothelium (E). Intact juxtaglomerular apparatus (JGA).Water retention in the Tubules (T). (photomicrograph x400)

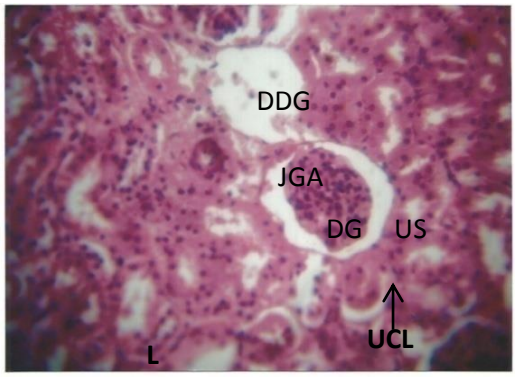


PLATE 6: a cross section of the renal cortex showing depleted glomeruli (DDG), wider urinary space (US), affected juxtaglomerular apparatus (JGA) and urinary tubules (UT). (photomicrograph x400)

LIVER

Plate 7 Control: the photomicrograph (x 400) of the sections of the liver in the control group showing the central vessel (CV) that are irregular in shape. The sinusoids (S) partly radiates from the vessels. The hepatocyte (H) appeared in cords and are multinucleated(N). The nuclei were open faced as can be observed in the normal liver sections.

Plate 8 MSG: Photomicrograph of the section of the hepatic lobule showing slightly enlarged central vessel (CV) with eroded endothelial (E) layer. The lumen of the vessel were also occupied by metasized tissues (MT) of the hepatocytes. The sinusoids (S) radiates out are blocked by hepatocytes (H). The hepatocytes appeared depleting of its cytoplasm content (CC). the nuclei of the organ were degenerating in nature as majority appeared tiny.

Plate 9 Viamin E: Photomicrogrph of the section of the hepatic lobule showing its central vessel whose endothelium is intact as slightly thickened. The sinusoid radiates from the vessels and are occluded by the sinusoids. The cytoplasm of the hepatocytes appeared depleted around the nuclei. There were slightly lipid deposits or accumulation of fat in the organ.

Plate 10 MSG+ vitamin E: Photomicrograph of the section of the hepatic lobule showing its enlarged vessels with intact endothelia linings. The sinusoids were occluded by the hepatocytes whose cytoplasm appeared depleted. The nuclei here also were enlarged. Also there were fatty accumulation here too.

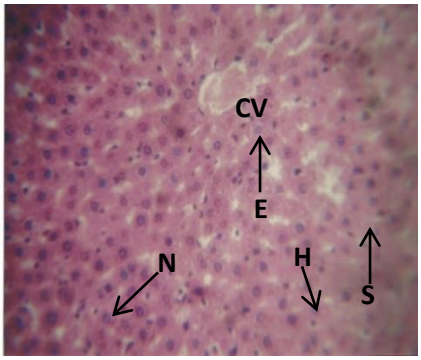


PLATE 7: a cross section of the control group of the hepatic lobule showing normal central vessel (CV) with fluid endothelium (E), hepatocytes (H), sinusoids (S) and nuclei (N). (Photomicrograph x400)

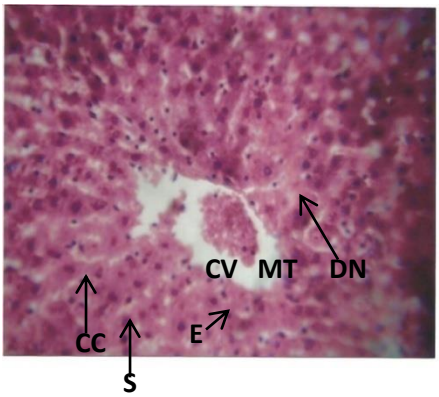


PLATE 8: a cross section of the MSG group of rhe hepatic lobule showing enlarged central vessel (CV) with eroded endothelium (E). Occupied lumen with Mesta sized tissue (MT), blocked sinusoids (S) and depleting cytoplasm content (CC). (Photomicrograph x400)

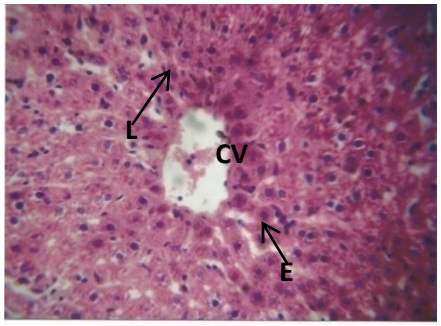


PLATE 9: a cross section of the vitamin E group of the hepatic lobules showing intact central vessel (CV) with its endothelium (E).(photomicrograph x400)

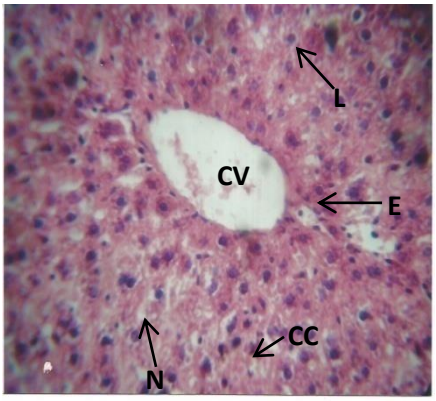


PLATE 10: a cross section of the MSG and vitamin E group showing enlarged central vessel (CV) with intact endothelium (E), depleted cytoplasm content, enlarged nuclei and accumulation of fat. (Photomicrograph x400)

DISCUSSION

In the study, we looked at the possibility of MSG to induce oxidative stress at the dose of 0.6mg/g body weight. The modulatory effect of vitamin E, a nutritional antioxidant on MSG-induced oxidative stress was also studied at the dose of 0.2mg/g.
Comparison of the final body weight with the initial body weight in each group revealed a significant difference among groups, hence, group 2 having the highest weight as compared with the control and vitamin E (**Table 4**). MSG intake could induce an increase in energy intake [22] which could lead to obesity [23] or alter the levels of carbohydrates, lipids and protein in rats [24]. In controlling of the body weights in MSG and vitamin E treated animals in group 4 reflects the possible role of vitamins which could lead to change in functions against MSG oxidant effect of the organs that are regulated by the nervous system and metabolic activities [25].

A significant increase in the liver and kidney weight of the rats was observed after administration of MSG (**Table 1**). Thus, could be attributed to increase in activity of inflammatory agents that could have resulted to inflammation of liver and kidney tissues [26].
Vitamin E reduced the MSG induced increase in the liver and kidney weight via its action as a radical scavenger. By scavenging the radicals that contributed to oxidative stress induced by MSG, vitamin E could help in reducing inflammation [14]. The changes in relative liver and kidney weights have been accompanied with changes in functional aspects.

Table 2 shows the activities of ALT and AST enzyme that were measured in serum samples. Significant ($p<0.05$) increase in the serum ALT and AST was observed in the MSG-treated rats compared to the vitamin E treated rats.
ALT enzyme is a sensitive marker of the liver damage [27]. Therefore, the increase in the serum ALT activity might perhaps be indication of the liver damage. MSG could dissociate easily to release free glutamate. The possible ammonium ion overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver, consequently releasing the ALT enzyme that may lead to its observed elevation. This increase could be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage [28].

The result seemingly agrees with the reports of Farombi and Onyema [29,14] that the activity of the serum ALT increased in male rats that were fed MSG probably due to the finding that MSG induced oxidative stress in the liver. Thus, it could be concluded that MSG may be hepatotoxic at a low dosage, hence, should be avoided during the treatment of liver disorders. Furthermore, since ALT

was a strong positive indicator of insulin resistance, diabetes mellitus and obesity [30] which are the risk factors for coronary heart disease [31]. The use of MSG even at low doses should not be encouraged because of the possible health implications. An increase in the activities of these enzymes indicates an effect due to the dose.

Administration of vitamin E found to have effect in improving in the serum level of ALT enzyme. This result agrees with the published studies where pre-treatment with vitamin E has been reported to confer protection against such changes in MSG induced-hepatotoxicity and oxidative stress in rats (13, 14] as shown in Table 2

The concentration of total protein, total bilirubin, globulin and albumin in the serum can be used as indicators for the state of the liver and can be used to differentiate between different types of liver damage. The observed reduction in albumin, total protein, globulin and bilirubin concentrations in serum (**Table 2**) indicate liver damage [32], arising from the intake of chemical compound. This may be an indication of diminished synthetic function of the liver which may consequently lead to enhanced retention of fluid in the tissues spaces.

Blood serum samples of the rats treated with MSG contained nominal decrease of urea as compared with urea nitrogen mean values of the control (**Table 3**). Mean serum urea of the control animals was 10.23mmol/L and that of MSG treated rats was 8.49mmol/L. The nominal reduction in serum urea concentration throughout the experimental period may be attributed to impairment of the urea cycle leading to reduced production of the metabolic product. Urea is the major nitrogen-containing metabolic product of protein catabolism. Serum urea nitrogen is a measure of renal function. Normally, the serum urea nitrogen level rises in heart failure, dehydration, or high protein diet and low urea nitrogen level can be seen in liver and renal damage or in liver diseases [33]. The significant increase in creatinine content of the serum following the administration of MSG may be attributed to compromise of the renal functional capacity.

The study showed that toxic effects of MSG on glomeruli of the kidneys were focal and segmental. There was shrinkage of glomeruli, increased cellular proliferation, and exudation of contents of capillaries with obliteration of their lumen. This is quite different from the control group. This finding are similar to those reported by Salam and Agha [34] who treated the rats with 2-3mg/g body weight. They also stated the renal proximal convoluted tubules were more severely affected than other tubules with inflammatory infiltration and focal hemorrhagic areas. Eweka and Igbibi [35] working on kidney of adult wistar rats observed distortion of renal cortical structures with some degree of cellular necrosis due to MSG. vitamin E administered did not show any positive result but quoted, it might be as a result of the dosage that are not even i.e 0.6mg/g of MSG as against 0.2mg/g of vitamin E or as a result of the nature of the vitamin used which is fat soluble that is capable of contributing to further damage since it was consumed by the rats daily. The group treated with only vitamin E showed water retention which invariably contained some lipids, proved that the vitamin E which is a good antioxidant though, should be consumed sparingly to prevent some hyperactivity of the vitamin in the body.

In the study, the liver of the animals administered MSG showed changes in the histological pattern evident by the enlarged central vessel with eroded endothelial layer, blocked sinusoids, depleting cytoplasmic content and degenerating nuclei compared with the control. Quite a few reports on the alteration in the liver histology have been documented although those studies used doses that were way above the dose we chose for this study [36, 37, 38, 39). The animals treated with vitamin E (MSG + vitamin E) showed a positive result having altered some effects as compared with the animals treated with MSG. It was observed that there were presence of fat deposits which was also observed in the animals treated with vitamin E only, this can be said to be caused by the nature of the vitamin being a fat soluble vitamin.

CONCLUSION

This results of the present investigation has shown that MSG at low dose is capable of producing alteration in the body weight, liver and kidney functions, liver and kidney weights and the histology. These alterations appeared in the liver and kidney because these organs are mainly responsible for detoxification of foreign compounds in the body. Vitamin E has shown to protect and restore the liver and kidney capabilities in inhibiting oxidative stress but when consumed appropriately to avoid hyperactivity of the vitamin.

RECOMMENDATIONS

1. **Read the labels**: MSG is a food ingredient; therefore it would appear in the list of the ingredients which are identified in decreasing order. Also, look for the presence of hydrolyzed vegetable protein or hydrolyzed plant protein. MSG will most likely be found near the bottom of the ingredients list.
2. **Use in moderation**: MSG does not perk up the flavor of fruit, fruit juice, candy, sweet baked goods, milk and butter. For those foods that benefit from its use, such as

vegetable and meat dishes, a general guideline is to allow no more than 5ml (5ml is equivalent to one teaspoon) per kilogram of food or 2ml per six servings of vegetables.

3. **Avoid adding MSG to commercially–prepared foods**: since many pre- packaged foods already contained MSG, further addition should not be necessary.

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