A HISTOLOGICAL STUDY OF THE HEPATIC AND RENAL EFFECTS OF SUBCHRONIC, LOW DOSE ORAL MONOSODIUM GLUTAMATE IN SWISS ALBINO MICE.

Adejoke Yetunde ONAOLAPO [1], Olakunle James ONAOLAPO [2] , Tolulope Josiah MOSAKU [1]

Onigbinde, Oluwanisola Akanji[1] and Oyeleke Abiodun[1]

ABSTRACT

The effects of low dose, subchronic oral monosodium glutamate (MSG) on the histology of the liver and kidneys were studied. This was to determine if MSG consumption at these doses is associated with histological evidence of hepatic or renal injuries. Forty adult Swiss albino mice weighing between 20-25 mg were assigned into 4 groups A, B, C and D. Group A served as control and received normal saline while groups B, C and D received MSG daily at 0.5, 1.0 and 1.5 mg/kg dissolved in normal saline respectively for 28days. On day 29 animals were sacrificed; liver and kidneys removed were processed for histological study. Statistical analysis was by one way ANOVA followed by a posthoc test, results were expressed as mean ±S.E.M. MSG consumption is associated with a dose- dependent albeit non statistically- significant increase in body weight compared to control, significant increase in relative liver weight occurred at 1 and 1.5 mg/kg and significant increase in relative kidney weight at 1.5 mg/kg. Liver and kidney histology showed loss of normal liver architecture with varying degrees of disorganization and apoptotic cell death, contraction of the renal glomerulus and thickening of the walls of the renal tubules. The study concluded that MSG at low doses causes hepatic and renal injuries.

Keywords: Glutamate, Anatomy, Pharmacology, Morphology.

1.0 INTRODUCTION

Monosodium glutamate (MSG) is a naturally occurring sodium salt of glutamic acid which was initially synthesized from wheat gluten but now produced in commercial quantities by bacterial fermentation (Leung and Foster, 2003). It is an important building block of protein (Pasquini and Berardelli, 2009). MSG is found in some quantity in many natural food substances and as either an additive and flavour enhancer in many commercially packed food products. MSG is useful in both home and restaurant cooking and it is a common component of Asian diets (Walker and Lupien, 2000). The unique flavour and taste of this compound has been categorized and established as a separate taste sensation "*umami"* taste (Ikeda, 1909). It is marketed in Nigeria as *Ajinomoto,* other trade names include: Vetsin, Accent and Tasting powder. MSG is composed of white colourless odourless crystals that exist in two forms called enantiomers although only the L forms are used as flavouring agents (Leung and Foster, 2003).The liver plays an important role in the metabolism of glutamate , some glutamate is converted here into lactate while the kidney takes part in its elimination although some MSG is metabolised by conversion into alanine in the intestinal mucosa (Garattiini, 2000).

MSG is ingested daily either as a component of naturally occurring food substance or as a food additive although only about $^{1}/_{1000}$ of the total mass of glutamate present in our tissues comes from exogenous glutamate sources (Hodgson, 2001). Daily dietary composition of glutamate varies from one race to another, however daily oral consumption ranges from 0.5 mg/kg amongst Americans and over 3g/kg in Taiwanese diets (Zhou *et al*., 2003; He *et al*., 2008; Shi *et al*., 2010), the quantity of MSG consumed by Nigerians we believe would fall somewhere between 1-2.5 g/day, Most of the glutamate found in our diet is from natural sources usually a diet rich in protein (Hodgson, 2001).

Recent reports unequivocally support the school of thought that there is no difference between the MSG added to foods and the glutamate that occurs naturally in foods (FASEB, 1995).

Up until recently most reports on the possible adverse reactions to MSG in the literature have been from case reports and not experimental studies, with most symptoms being transient and not life-threatening, there however has been some documentations on the various effects of MSG on organ systems although this studies have used MSG at doses significantly higher than the daily average consumptions /person.The neurotoxic effects of MSG was first demonstrated in 1957 by Lucas and Newhouse (Lucas and Newhouse, 1957), they observed retinal degeneration that was a sequelae of intravenous injection of MSG in infant mice. Several other studies have also reported severe neuropathological effects of MSG on animals at significantly high doses. Park and his colleagues (Park *et al*., 2000) reported significant injury involving the neurons in the arcuate nucleus and hypothalamus following the administration of a single intraperitoneal injection of MSG, impaired memory retention in adult mice was also noticed . González-Burgos reported two important effects resulting from neonatal exposure to MSG the first was an initial excitotoxicity that resulted in cell death followed by a neuroprotective effect which saw the proliferation of glial cells and the subsequent uptake of glutamate hence favouring survival of the remaining neurons (Gonzalez-Burgos *et al*., 2001). Studies have also demonstrated deleterious effects on the cerebellum evidenced by tremor, unstable and uncoordinated movements and ataxia as well as varying degrees of renal injury following consumption of high doses of MSG (Eweka, 2007). Research on the safety of MSG has undergone rigorous review by scientific advisory bodies and various national governments. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation in 1987 declared L-glutamate safe by arriving at an "Acceptable Daily Intake (ADI) not specified" this was also reaffirmed in 2004 (JECFA, 1987;

JECFA, 2004). The term "ADI not specified refers to the total dietary intake of glutamates that is as a result of their use at the quantitity necessary to achieve the desired effect as food additives, and from the normal naturally occurring quantity in food is not hazardous to health (JECFA, 2004).

During an earlier study on the neurobehavioural effects of MSG(Onaolapo and Onaolapo, 2011), some histological changes were noticed in the liver and kidneys of some of the animals randomly selected necessitating a full evaluation of its effect on liver and kidney microanatomy at doses well below those known to be toxic.

2.0 **MATERIALS AND METHOD**

2.1 EQUIPMENTS AND APPARATUS

Electronic precision balance, plastic animal cages, sterile disposable syringes (1, 5, and 10 ml) and needles and cotton wool.

2.2 REAGENTS AND DRUGS

Normal Saline, 99% monosodium glutamate (Ajinomoto brand) was purchased from the market, weighed and dissolved in measured volume of isotonic saline solution to get desired concentrations. MSG at the varying doses (0.5, 1.0 and 1.5 mg/kg) (Onaolapo and Onaolapo, 2011) was administered orally using a cannula.

2.3 ANIMALS

Healthy adult Swiss albino mice purchased from the Empire Animal farms in Osogbo, Osun State, Nigeria were used. The animals weighed between 20 and 25 g. After being weighed on an electronic balance, the animals were randomly divided into four treatment groups. The animals were housed in plastic cages measuring 16"x12"x10" (10 mice in each cage). All animals had free access to food and water *ad libitum*. They were maintained under standard

laboratory conditions that is a well aerated room with alternating light and dark cycles of 12 h each and at room temperature of 25°C. The experimental protocol was approved by the Ladoke Akintola University Animal Ethics Committee. All rules applying to animal safety and care were observed.

2.4 EXPERIMENTAL METHOD

This research work was carried out between October and November 2011 in the Histology laboratory of the department of Anatomy, Ladoke Akintola University of Technology Ogbomosho. Forty animals were used for the experiment. The animals were randomly assigned into four groups A, B, C and D. Group A was the control and received normal saline. Groups B, C and D received MSG orally at 0.5, 1.0 and 1.5 mg/kg respectively for a period of 28 days. Animals were weighed weekly using a Mettler weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland). At the end of the experimental period rats were observed for changes in their physical characteristics and then sacrificed by cervical dislocation and the liver and kidneys of each of the animals dissected out through a midline abdominal incision passing through the abdominal wall musculature into the peritoneal cavity. The organs were observed grossly and then fixed in 10% formolsaline for histological studies. Paraffin sections were cut and stained with Haematoxylin and Eosin for general histological study. An Olympus BX50 digital light microscope was used to examine the slides and acquire photomicrographs.

2.5 STATISTICAL ANALYSIS

All behavioral data were analyzed using the one way analysis of variance (ANOVA) followed by post hoc tests (Student Newman Keul's) carried out to determine the source of a significant effect. Results were expressed as Mean ± S.E.M., $p<0.05$ is taken as accepted level of significant difference from control.

3.0 RESULTS

3.1 THE EFFECTS OF MONOSODIUM GLUTAMATE ON BODY WEIGHT

Figure 1 represents the mean body weight taken weekly over the 28 day period. Result showed progressive increase in the body weight in all treatment groups. There was however no significant difference in growth proportion within the groups throughout the experimental period. Comparison of the final body weight with the initial body weight in each group revealed a dose related decrease in percentage weight gain in the groups that received MSG. The animals in groups B, C and D had a percentage weight change of 21.25, 15.88 and 12.06% respectively compared to control group (23.37%). These differences were however not significant.

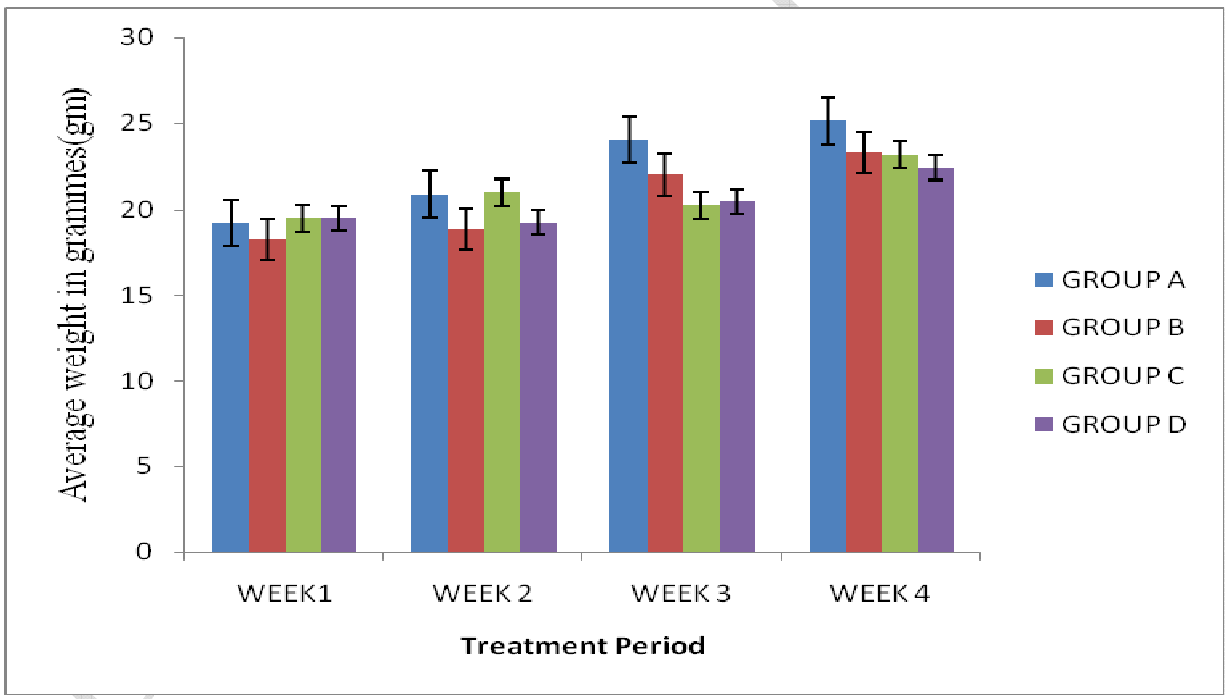

Figure 1: Effect of MSG (0.5, 1.0, 1.5 mg/kg) on weekly body weight. Each bar represents Mean ±S.E.M,* $p \leq 0.05$ compared to the control, n=10. A control, B, C and D received MSG at 0.5, 1.0, 1.5 mg/kg respectively.

3.2 THE EFFECTS OF MONOSODIUM GLUTAMATE ON RELATIVE LIVER AND KIDNEY WEIGHT

Figure 2 represents the mean relative liver and kidney weight following administration of MSG. There was a significant dose dependent increase in relative liver weight in the groups that received MSG at 1 and 1.5 mg/kg respectively compared to control while the relative kidney weight increased significantly at a dose of 1.5 mg/kg compared to control.

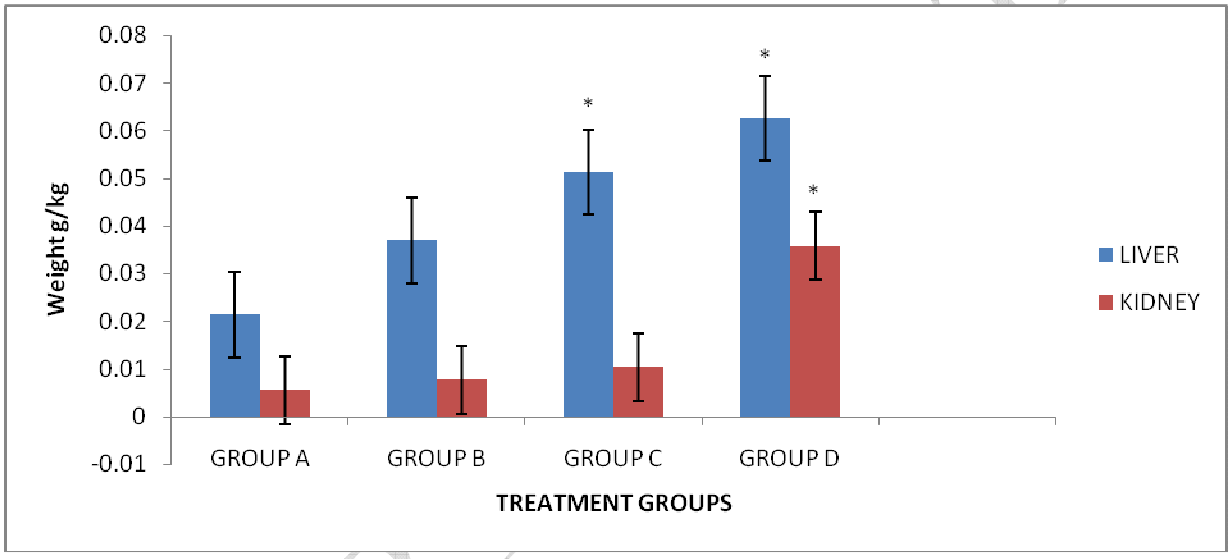

Figure 2: Effect of MSG (0.5, 1.0, 1.5 mg/kg) on the mean relative liver and kidney weight. Each bar represents Mean ±S.E.M,*p ≤ 0.05 compared to the control, n=10. A control, B, C and D received MSG at 0.5, 1.0, 1.5 mg/kg respectively

3.3 THE EFFECT OF MONOSODIUM GLUTAMATE ON LIVER MICROANATOMY

Sections through the liver of group A animals (Plate 1), showed sheets of radially arranged hepatocytes with well demarcated nuclei and intervening sinusoids, normal central vein and hepatic artery, features in keeping with normal histology. Examination of the sections through livers of animals in groups B (plate 2), C (plate 3) and D (plate 4) showed loss of liver architecture with varying degrees of liver parenchymal disorganization, cell death, dilation of the

central vein and presence of inflammatory cells within and around the central vein there were also variations in the sizes and shapes of the nuclei, vacuolation and pyknosis (Plate 1).

3.4 EFFECTS OF MONOSODIUM GLUTAMATE ON KIDNEY MICROANATOMY

Sections through the kidneys of group A (Plate 5) animals showed normal kidneys with well demarcated cortex, medulla, normal Bowman's capsule and glomerulus as well as normal sized renal tubules. The sections of kidneys of groups B (plate 6), C (plate 7) and D (plate 8) animals showed widening of the Bowman's space due to contraction of the renal glomerulus and hypercellularity

4.0 DISCUSSION

Monosodium glutamate is consumed in considerable amounts in almost all forms of foods in Nigeria. MSG is one of the most extensively researched food additives in the world (JECFA, 2004; FASEB, 1995). Results of studies continue to support the finding that at levels normally consumed as a flavor enhancer, MSG is safe for the general population (Hodgson, 2001). In this study we evaluated the effect of subchronic oral MSG on the body weight, relative liver and kidney weight and liver and kidney morphology in mice. The results of our study revealed that at the doses of MSG tested, there was a dose related increase in body weight although the percentage weight gain reduced with increasing doses of MSG, the weight increase observed in the control group however was slightly higher compared to the MSG treatment group. A number of studies have examined the potential link between MSG and body weight. There have been speculations that people tend to eat larger helpings of food with MSG because it just tastes better than they would if the food did not contain MSG. Another school of thought suggests that MSG might interfere with signaling systems that regulate appetite centres also up scaling food consumption and hence weight gain initially and possibly obesity with chronic

consumption (Bergen e*t al*., 1998; Mozes *et al*., 2004; Kawakita *et al*., 2005; Inuwa *et al*., 2011; Bhattacharya *et al.,* 2011; Tawfik and Al-Badr 2012).

A significant increase in liver and kidney weight was observed following administration of MSG These could be as a result of increase in inflammatory activity with resultant tissue oedema, some other studies have also documented this at higher doses of MSG (Tawfik and Al-Badr 2012). In this study the liver of experimental animals showed changes in histological pattern evident by disruption of hepatic cords, presence of inflammatory cells within and around the central vein with uneven sizes of nucleus in hepatocytes. Quite a few reports on alteration in liver histology and/or biochemistry have been documented although this studies used doses that were way above the dose we chose for this study (Eweka and Om'Iniabohs 2011; Inuwa *et al*., 2011; Egbuonu *et al*., 2009; Ortiz *et al*.,2006). These results would mean that subchronic administration of oral MSG results in alteration in the hepatic structure that are comparable with those of studies that used doses that were at least 100-1000 times the doses in this study

Kidney microanatomy in groups that received MSG (B, C and D) compared to controls showed dilatation of the Bowman's space, contraction of the renal glomerulus and hypercellularity which are in keeping with renal injury, this corroborates results of studies carried out in 2007 by Eweka (Eweka, 2007). He investigated the effects of MSG on the kidney of adult Wistar rats given 3g and 6g of MSG thoroughly mixed with growers mash for the period of fourteen days, results of kidney microanatomy showed varying degrees of cytoarchitectural distortion and reduction in the number of renal corpuscles in the treated groups which was at variance with that of the control group. Ingestion of MSG resulted in cellular necrosis of the Bowman's capsule, at a dose of 6 g degeneration and atrophy of the kidneys were seen, he concluded that high doses and chronic ingestion of MSG resulted in the degenerative and atrophic changes observed in the renal

corpuscle, although we used lower doses of MSG than was used by Eweka the progressive renal injury at increasing doses of MSG was evident.

The effects observed in both the liver and kidneys could have occurred because these organs are involved in the metabolism of glutamate or as in another study it may be due particularly in the liver exacerbation of trans-fat induced fatty liver disease in mice by a mechanism that includes increased central adiposity and alterations in both hepatic and white adipose tissue gene expression (Collison *et al*., 2009). MSG has been reported to increase oxidative stress and some studies have also documented amelioration of the hepatotoxic or nephrotoxic effects by the administration of radical scavengers such as vitamin E or C (Faronmbi and Onyeama, 2006; Onyema *et al.* 2006). The risk of hepatic or renal injury may have been increased because MSG was administered as a bolus, this school of thought is supported by Takasaki *et al*., who while studying the mechanisms by which glutamate induced brain injury came to the conclusions that in order to produce neurotoxic effect in infant mice MSG has to be given not only in relatively high concentration but also as a bolus solution (Takasaki, 1978).

5.0 CONCLUSIONS

This study concluded that MSG at doses tested resulted in varying degrees of liver and kidney injury. It is important to note that all doses of MSG used in most published studies were very high and if MSG at the doses we have studied showed some evidence of organ injury, and then more research needs to be conducted to verify the safety profile of this widely used food additive.

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