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Monosodium glutamate-induced changes on plasma markers of pancreatic function in adult male Wistar rats

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Abstract

Monosodium glutamate (MSG), one of the most widely used food additives worldwide, has been associated with harmful effects on different organs in animal models and human clinical volunteers. The present study evaluated the median lethal dose (LD_{50}) and the effect of sub-chronic MSG consumption on plasma markers of pancreatic function in adult male Wistar rats. Seventy-six adult Wistar rats weighing 200 ± 50 g were randomly assigned into three groups viz: LD_{50}, n=12, MSG-treated, n=32 and non-MSG-treated control, n=32. At weekly intervals, blood was collected from four randomly selected rats in each group for plasma markers (glucose, insulin, lipase and amylase) assays. Morbidity and mortality were not observed in the LD_{50} group. The glucose, insulin, lipase and amylase levels of MSG-treated group were significantly ($P < 0.05$) higher than those of non-MSG-treated control group. These findings suggest that MSG caused changes in the plasma activities of lipase and amylase, the absence of gross and microscopic lesions of congested blood vessel point to possible physiochemical alterations in the pancreatic acini with consequent enzyme leakage.

Keywords: LD_{50}, Monosodium glutamate, Pancreas, Plasma, Wistar rats

Introduction

Monosodium glutamate (MSG), a sodium salt of glutamic acid, is used as a flavor enhancer in food industry (Walker & Lupien, 2000; Boonnate *et al.,* 2015). MSG contains 78% of glutamic acid, 22% of sodium and water. Glutamic acid is one of the most abundant amino acids found in nature and the main component of many proteins and peptides of most tissues (Ibukun *et al.,* 2015). It is produced in many countries around the world through a fermentation process of molasses from sugar cane or sugar beets, as well as starch and corn sugar (Enameli & Danielson, 2014). MSG is sold in most open market stalls and stores in Nigeria as "Ajinomoto" marketed by West African Seasoning Company Limited; as "Vedan" or "White Maggi" by Mac and Mei (Nig.) Limited. It is commonly consumed as food additive in both household and restaurants (Michael & Peter, 2015). Locally and globally, there have been contradictory reports concerning the safety of this food additive (Eweka *et al*., 2011). Both animal model experiments and human clinical reports have suggested its harmful effects when consumed overtime (Ibukun *et al.,* 2015). In Nigeria, despite epidemiological studies report on the negative consumer response to MSG (Inuwa *et al.,* 2011), reputable international organizations like the Food and Drug Administration (FDA) and National Agency for Food and Drug Administration and Control

(NAFDAC), as well as nutritionist have continued to endorse that MSG is safe as a flavor enhancer, without any adverse reactions in humans (Eweka *et al.,* 2010). Nonetheless, consistent metabolic effects of MSG have been demonstrated in animal studies including pancreatic pathology in these models (Sasaki *et al*., 2009).

The pancreas is a retroperitoneal organ critically important for intestinal digestion of food. Most of the pancreas consists of the exocrine glands that synthesize and secrete a great majority of digestive enzymes into the pancreatic duct tributaries and into the duodenum (Mohamed & El-Mandrawy, 2016). Pancreatic lipase is the main enzyme responsible for digestion of dietary triglycerides. Glucose is the major source of energy used by the cells. However, glucose cannot enter the cell unless in the presence of insulin (Farhood *et al.,* 2014). In a normal physiologic function of pancreas, the right amount of insulin is produced to transport glucose into the cells. In pathological pancreas, little or no insulin is produced, or the body cells do not respond to the insulin that is produced leading to accumulation of glucose in the blood or elevation of its levels (hyperglycaemia) resulting in diabetes mellitus (Emanuele *et al.,* 2009). Despite these lines of evidence, there is paucity of information on detailed markers of pancreatic function with respect to MSG toxicity; therefore the aim of the present study was to evaluate the biochemical changes in the pancreas of adult male Wistar rats following prolonged levels of oral MSG supplementation.

Materials and Methods

This study was scrutinized and approved by the Ahmadu Bello University Committee on animal use and care (ABUCAUC).

Study location

The study was conducted at the Department of Veterinary Pathology, A.B.U Samaru, Zaria, Kaduna State, Nigeria. The area is located within the Northern Guinea Savannah Zone of North Western Nigeria. It lies between latitude 7^0 and 11^0 N and longitude 7^0 44 E. It has an average rainfall of 1,000 to 1, 250 mm and an average temperature of 17 ^{0}C to 33 ^{0}C and vegetation made up of predominantly trees and grasses (Abbas , 2012).

Animals

Seventy-six adult male Wistar rats aged 10-12 weeks and average weight of 200 ± 50 g were obtained from animal breeding unit, Department of Human Anatomy, Faculty of Medicine, A.B.U, Zaria, Nigeria and used for the study. Rats were housed in aluminum cages covered with wire mesh under normal light and temperature controlled environment, at the laboratory animal pen house of Department of Veterinary Pathology, A.B.U, Zaria and allowed to acclimatize for 2 weeks before commencement of the study.

The median lethal dose (LD_{50}) determination

The median lethal dose (LD_{50}) of MSG was carried out using a standard Two–phase approach as described by Lorke, (1983). This comes in 2 phases: phase 1 consists of 9 adult rats divided into 3 groups of 3 experimental animals each. Groups 1, 2, 3 were dosed 10 mg/kg, 100 mg/kg and 1000 mg/kg, respectively. The animals were observed for clinical signs of toxicity for 48 hours and then subsequently for 2 weeks. The result obtained from phase 1 was then used to determine phase 2 which consisted of 3 groups of 1 animal each and were dosed with 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of MSG, respectively.

Experimental design

The rats were randomly assigned into two groups of 32 each viz: MSG-treated rats and non-MSG-treated rats. Pelletized commercial grower feed (Vital feed®, Jos, Nigeria) and drinking water were provided *ad libitum*. Rats were kept under natural environmental conditions at the temperature of 24^0C - 27^0C and relative humidity of 70 - 80 %.

General care of the rats was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (IACU, 2002).

Monosodium glutamate administration

Monosodium glutamate (Ajinomoto brand, manufactured by Ajinomoto Company, incorporation Tokyo, Japan, marketed by West African seasonings Company Limited) was obtained in a dry form containing active ingredients. An aqueous solution was prepared daily by reconstituting 16 g of MSG in 32 ml of distilled water to obtain a concentration of 500 mg/ml. The MSG-treated group was administered MSG orally on daily basis at a dose of 5 g/kg body weight using a graduated syringe and a stainless steel intubation cannula at approximately the same time, while non-MSG-treated group was only administered distilled water as placebo

throughout the 8 weeks period the experiment lasted.

Blood sample collection and estimation of biochemical parameters

Four rats were randomly selected and humanely sacrificed from each group, at weekly intervals for the 8 weeks that lasted the experiment; three millilitres of blood was collected from the jugular veins and dispensed into ethylene diamine tetra acetic acid (EDTA)-impregnated sample bottle and allowed to stay for a while before centrifugation to obtained plasma. The harvested plasma samples were used for assay of plasma markers of pancreatic function. Glucose (mmol/l), insulin (mmol/L), lipase (U/L) and amylase (IU/L) levels were analyzed using test kits obtained from Reckon Diagnostic Private Limited, (3/7 Gorwa, Vadodara 390016, Gujarat, India) with a fully automated analyzer (Rayto Chemray-120 fully automated clinical blood chemistry analyzer, China, mainland) using standard methodology according to manufacturer's instruction.

Statistical analyses

Data generated from the study were subjected to students' t-test using Graph pad prism Version 5.00 for windows, (Graph Pad Software, San Diego, CA, USA) and values of $P \leq 0.05$ were considered significant. The mean ± standard deviation (SD) of the results obtained were calculated and presented in figures (via bars).

Results

Effect on plasma insulin levels

The plasma insulin levels of the MSG-treated group were significantly ($P < 0.05$) higher than that of non-MSG-treated control from weeks 5 to 8 post-treatment (Figure 1).

Effect on plasma glucose levels

The plasma glucose levels of the MSG-treated group were significantly ($P < 0.05$) higher than that of non-MSG-treated control from weeks 3 to 8 post-treatment (Figure 2).

Effect on lipase levels

On weeks 5 to 8 post-MSG treatment, the plasma lipase levels of the rats in MSG-treated group were significantly ($P < 0.05$) higher than that of group non-MSG-treated control (Figure 3).

Effect on amylase levels

The plasma amylase levels of MSG-treated group were significantly ($P < 0.05$) higher than that of the non-MSG-treated control from weeks 3 to 8 post-treatment (Figure 4).

Gross and microscopic findings

Post-mortem examination of the MSG-treated and control rats in this study showed no apparent gross lesions in any of the organs including the pancreas.

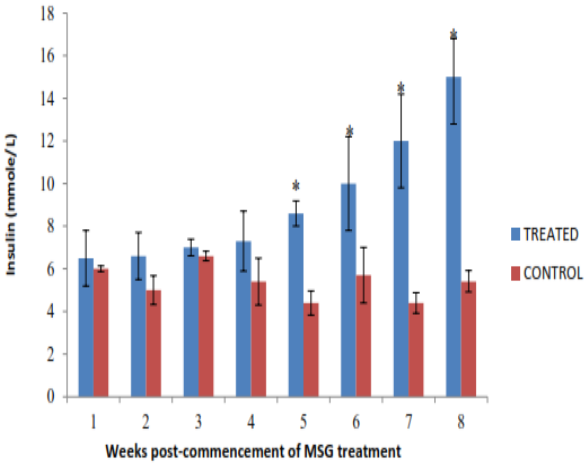

Figure 1: Mean (± SD) plasma insulin concentration of MSG-treated and control rats. Means with superscript * differs significantly from their corresponding control values

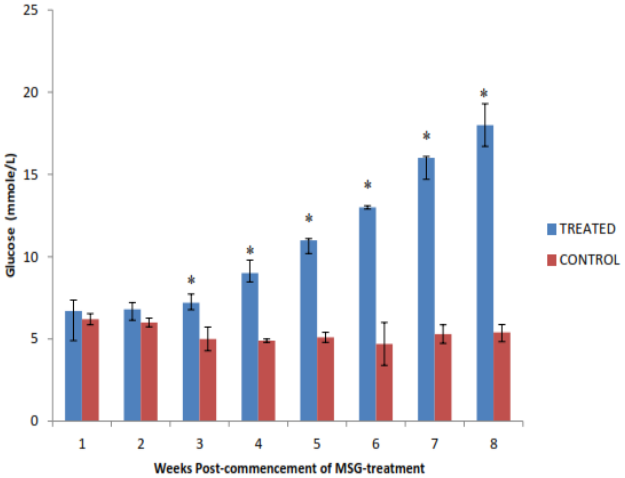

Figure 2: Mean (± SD) plasma glucose level of MSG-treated and control rats. Means with superscript * differs significantly from their corresponding control values

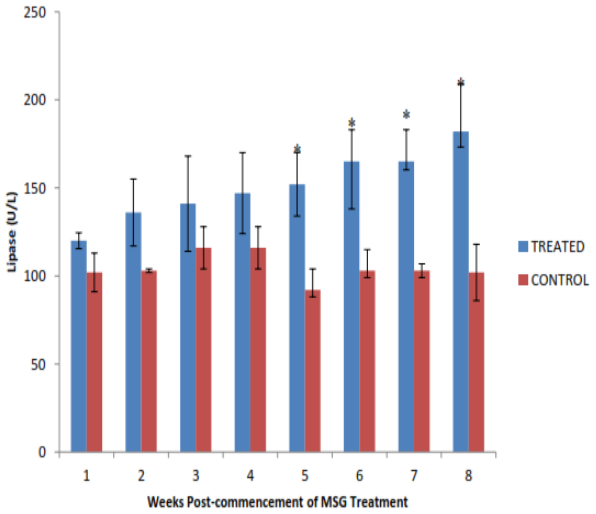

Figure 3: Mean (± SD) plasma lipase activity of MSG-treated and control rats. Means with superscript * differs significantly from their corresponding control values

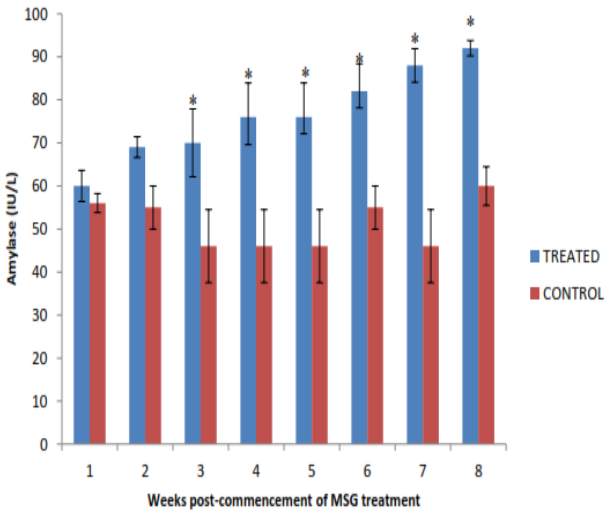

Figure 4: Mean (± SD) plasma amylase activity of MSG-treated and control rats. Means with superscript * differs significantly from their corresponding control values

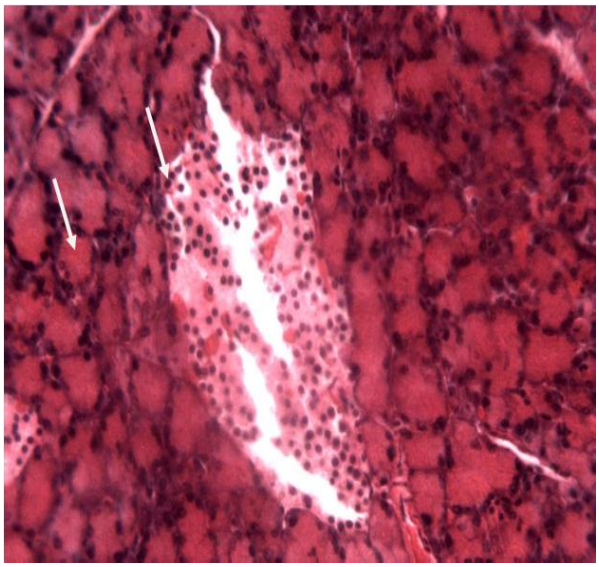

Plate I: Photomicrograph of the pancreas of week eight control (non MSG-treated) male Wistar rat showing pancreatic acinar and β-islet cells (arrows). H & E Stain x 250

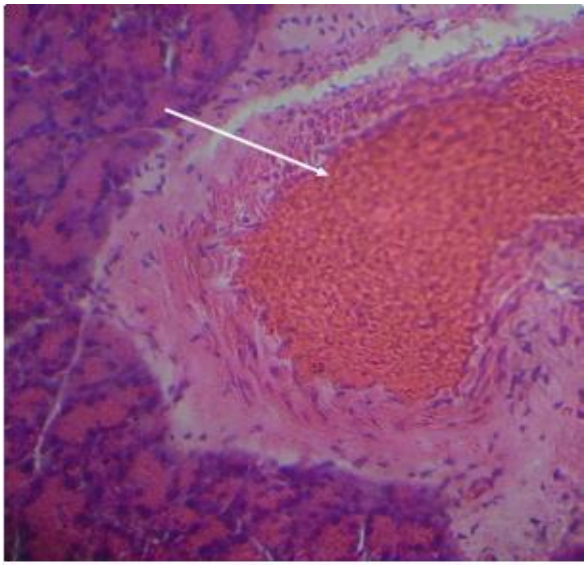

Plate II: Photomicrograph of the pancreas of week eight MSG-treated male Wistar rat showing congestion of blood vessel (arrow). H & E Stain x 250

Histopathology examination revealed congestion of blood vessels of the pancreas. The β-islets cells of Langerharns showed no hsitopathologic changes in both MSG-treated (plate I) and non-MSG-control rats (plate II).

Median lethal dose (LD_{50})

There was neither morbidity nor mortality in the three rats in each group treated with MSG at 10, 100 and 1000 mg/kg within 48 hours post treatment. This was then followed by second phase which consisted of 3 groups of one rat each at 1600, 2900 and 5000 mg/kg, respectively. There was neither morbidity nor mortality 72 hours post treatment.

Discussion

Several literatures have reported that the oral dose of MSG that is lethal to 50 % of subjects (LD_{50}) in rats and mice is 15,000-18,000 mg/kg body weight (Walker & Lupien, 2000). This value was however,

criticized as a parameter for toxicity assessment for MSG (Oriaghan *et al.,* 2012). But even so, the finding gave clue on the range of doses that were used in subsequent toxicity testing (Aniagu *et al.,* 2004). The present study showed that at dose of 5 g/kg, there was neither toxicity sign nor mortality observed when monitored for 48 hours post-treatment according to Lorke's method.

The findings in the present study of significantly higher plasma insulin levels in the MSG-treated group of rats suggested that, MSG might be exerting some level of insignificant cytotoxicity effect on the β-cells of islets of Langerhans of pancreas. This corresponds to the findings in humans (Hugues *et al*., 2002) and in adult rabbits (Oriaghan *et al.*, 2012) in which oral administration of MSG was observed to increased insulin levels. However, it is also possible that MSG might have caused insulin resistance or decrease insulin sensitivity of the target cells or tissues, thus, leading to decreased glucose utilization, the increased level of which continued to trigger more release of the insulin from the pancreatic β-islet cells.

Contrarily, despite the increase in plasma insulin concentration, there was continual increase in plasma glucose levels in the MSG-treated group from week 03 and throughout the period of the experiment. This corresponds to the findings where significant increase in plasma glucose levels was reported with MSG altering the regulatory mechanisms that affect fat metabolism (Oriaghan *et al.,* 2012), resulting in the propensity for creating adipose tissue (Vice *et al.,* 2005) and the weakening effect of fats on insulin action (Guyton & Hall, 2006). Additionally, the increase in mean plasma glucose level observed could be linked to the impairment in glucose adipose tissue uptake earlier observed following MSG-exposure in rats due to significant decrease in glutamate 4 (GLUT4) receptor in fat cell (Macho *et al*., 2000), skeletal muscle, cardiac muscle and brown adipose tissue in mice thus resulting into decrease glucose utilization and its subsequent released into circulation (Machado *et al*., 1993). Furthermore, the observed increased in plasma glucose levels in MSG-treated rats may also be closely related to the neurotoxic and brain damaging effects of MSG earlier reported (Rascher & Mestres, 1980). Since the hypothalamus is involved in the regulation and action of insulin (Guyton & Hall, 2006), a hormone that metabolizes glucose, damaged to the brain cells following MSG-exposure may render them to become dormant to the blood glucose level and or the feedback regulatory mechanism may not function to regulate insulin secretion or action. Earlier research works on pancreatic function were mostly focused on endocrine abnormalities of the beta-cells; hyperglycaemia and abnormal lipid profile among others; while very little concern were paid to the pancreatic exocrine glandular cells (Ravisekar *et al.,* 2015), thus resulting into a dearth of information on pancreatic exocrine functions. The exocrine pancreatic acinar cells secrete both lipase and amylase; that function in the breakdown of lipids (fat) and starch, respectively. Serum or plasma lipase and amylase are generally measured to determine the functional efficiency of the pancreas (Seguna *et al.,* 2013). The observed increased in plasma levels of both enzymes may be attributed to the effect of oxidative injury or impaired membrane permeability of the acinar cells earlier reported (Azevedo-Martins *et al.,* 2003), which may result into release or leakage of the enzymes into blood circulation instead of passing through the duct to the intestine. Similarly, an increase in the levels of pancreatic enzymes may be secondary to an imbalance between pancreatic release and renal clearance (Buchman *et al.,* 1993), although liver and renal damage probably due to oxidative effect is suspected to play a role in inducing pancreatic hyper-enzymemia since the liver is suspected to be a major organ for amylase removal (Pezzilli *et al.,* 1999).

The only observed histopathological changes of congested blood vessels of the pancreas is contrary to earlier studies that demonstrated MSG-parenteral exposure causes various changes in pancreatic islets such as haemorrhages (Boonnate *et al.,* 2015), hypertrophy, hyperplasia (Sasaki *et al.,* 2009), decrease in acinar cells and fibrosis (Nakayama *et al.,* 2003). However, these conditions may not represent the true scenario for oral exposure and such could be a reason for variation in our finding.

In conclusion, the observed alterations in the activities of plasma biochemical markers of pancreatic function in the MSG-treated rats in the absence of gross and microscopic lesions could be pointing to possible physiochemical alterations in the pancreatic acini with consequent enzyme leakage.

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