



Investigation of a child-equivalent dose of monosodium glutamate toxicity in Wistar rats.

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ABSTRACT

Children are more likely to consume monosodium glutamate (MSG) as this is prevalent in fast food such as fried chicken and snack food such as chip products. The aim of this study was to establish the toxic effects of higher MSG consumption in one-month old Wistar rats to estimate the human equivalent dose (HED) specific to children. To understand the impact of higher MSG consumption Wistar rats were fed orally four different doses (group I-IV: 2,000 mg/kg, 4,000 mg/kg, 20,000 mg/kg and 30,000 mg/kg which with respect to HED (9 years in children), were 480mg/kg, 960 mg/Kg, 4800 mg/Kg and 7200 mg/Kg) daily for 5 weeks compared with the control group. Histopathology was studied using Hemotoxylin and Eosin staining. The results showed an increased body weight in all MSG groups; colon with focal aberrant crypt hyperplasia noted in group IV and lymphoid cell aggregates noted in group III. The liver showed feathery degeneration and focal portal congestion were noted in all the MSG groups compared to the control groups. The kidneys showed focal congestion of blood vessels in all MSG groups. Stomach tissues exhibited no significant changes. Brain tissue sections showed congested blood vessels, and focal gliosis in group I and IV, ballooning degeneration was noted in group I, neuronal aggregates were found in group II, but group III showed no evidences of aggregates, gliosis or necrosis. This study suggests that regular and higher consumption of MSG had a deleterious impact on the organs as evident in the histology analysis and hence it is better to avoid consuming MSG containing foods especially for children.

KEY WORDS: Monosodium glutamate, MSG, human equivalent dose, HED, intestines, liver, kidneys, brain

INTRODUCTION

Human health mainly depends on the food they eat. Eating tasty and palatable food doesn't mean that it is nutritious food, instead it can lead to an imbalance in nutrition or overfeeding (1). Most commercial food items and bakery products have taste enhancers and flavoring agents to increase the taste and palatability of the food. Monosodium glutamate (MSG, E621) is one

such widely used taste enhancer and flavoring agent added into commercial food. The chemical formula of monosodium glutamate is $C_5H_8NaNO_4 \cdot H_2O$ and the molecular weight is 187.13 g/mol. MSG is freely soluble in water and appears as white odorless crystals or crystalline powder (2). In Asian countries, the demand for MSG has increased because of the change in dieting habits, increased urbanization and non-stop development in food processing (3). It has been estimated that the intake of MSG in Asian countries was about 4 g whereas in European countries it was about up to 1 g (4). However, there is no defined or

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restricted value for MSG consumption so far, and no safe limits have been published. In the absence of a safe limit, it could be possible to consume too much MSG which could lead to adverse effects to human health (5).

Food is digested and nutrients are absorbed in the gastrointestinal tract. The absorption of nutrients in the intestine is a crucial process in humans, with constant cell loss and replenishment in the intestinal epithelial surface (6). Interestingly, the MSG diabetes model renders mice more susceptible to azoxymethane-induced colorectal cancer (CRC) (7). MSG induces obesity and diabetes (both of which are linked to early onset CRC) in animal models (8-11). MSG was shown to be genotoxic to the human peripheral blood lymphocytes in an *in vitro* study demonstrating that, when compared to the control, MSG significantly and dose-dependently (250, 500, 1000, 2000, 4000 and 8000 mg/mL) enhanced the frequencies of chromosomal aberrations, sister chromatid exchange, and micronuclei in all MSG treatments. A further study investigated one hour exposure of MSG in human peripheral blood cells; DNA was isolated from the cells and was shown to have DNA damage in a comet assay (12).

Rat models are widely used in toxicology studies as their physiological, morphological and genetic features are close to humans. In addition, they have an advantage that facilitates multiple samplings due to larger body and organ size compared to mice. The present study used one-month old male albino Wistar rats, as at that age the rat age is equivalent to the age of a nine-year-old child (13). According to the World Health Organization's (WHO) toxicological evaluation of MSG LD₅₀, the value for rat was 19,900 mg/kg body weight (p.o) (14). Many different types of food, such as prepared meals and processed meat, such as bacon, pastrami, pepperoni, sausages, luncheon meat, smoked meat products, hamburger, cold cuts, and salami, contain MSG. Gravies and sauces such as ketchup, mayonnaise, regal sauce, salad dressing, soy sauce, and mustard may also contain MSG. Soup bases such as bouillon cubes and granulated maquillages, seasoned snacks such as potato and corn chips,

seasoning, spices, gelatin containing substances and bodybuilding protein may also contain MSG (15, 16). Many fast-food restaurants add MSG to enhance the flavor of everything from junk food nuggets, burgers and fries, as well as the seasoning used on some French barbecues (15).

Now-a-days, children are often exposed to MSG at a younger age due to changes in eating habits. Different levels of MSG in various food products might result in an increased MSG consumption. However, the MSG intake in children has not been established as it depends on the individual. Therefore, it would be valuable to understand how much MSG is consumed by children. Hence in this study, investigation of different MSG doses using Wistar albino rats and human equivalent dose (HED) conversion formula (17) was carried out so that the results could be applied to toxicological and drug development studies. HED could be defined as anticipating the dose of a chemical that produces effect in human equivalent to the effect as that observed in animals at a given dose (18). HED was calculated using the bodyweight and body surface area by employing the Km factor. Conversion of mg/kg dose into mg/m² (body surface area) dose relies on the body weight of animals.

Generally, body surface area is calculated using Equation 1.

$$\text{mg/m}^2 = \text{Km} \times \text{mg/kg} \quad \text{Eq. 1}$$

The Km factor varies from species to species and increases with increase body weight of the species. Thus, the Km factor could be calculated using Equation 2.

$$\text{Km} = \frac{100}{k \times w^{0.33}} \quad \text{Eq. 2}$$

Where the k value differs from species to species (19). For example, the Km factor value for rats = 6 and Km factor for humans = 25 (17).

The main objectives of this study were to first determine a standard human equivalent dose for MSG in children based on a rat model. Second, to study the effects of

MSG on different organs (colon, rectum, liver, kidney, stomach and brain) using histopathological analysis of tissue morphology of rats that had been fed MSG and comparing them to a control group of rats.

MATERIALS AND METHODS

Chemicals

Monosodium glutamate containing sodium salts of glutamic acid of 99.9% purity was purchased from the local market. It is commonly used as taste enhancer in fast-food centers and conventional restaurants. The MSG was dissolved in water and administered orally at different concentrations, except for the control group which did not receive MSG.

Experimental animals

Healthy, one month old male Wistar albino rats (60–70 g/kg) obtained from the animal house facility, Karpagam Academy of Higher Education, were used in the present study. The study was approved by the Institutional Animal Ethical Committee (KAHE/IAEC/2021/11-09/010) and committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India. The animals were maintained in polyurethane cages ($25 \pm 3^\circ\text{C}$) under healthy and sterile conditions as required for animal research. They were fed commercial food pellets and water (ad libitum) and maintained under 12-hour light and dark cycles.

Rat groupings and human equivalent dose (HED) calculations for MSG in animal models

HED for children was calculated using Equation 3 and the appropriate Km value.

$$\text{HED (mg/kg)} = \text{Animal dose (mg/kg)} \times \frac{\text{Animal Km}}{\text{Human Km}} \quad \text{Eq. 3}$$

Where, the human Km value for a child = 25 and for rats the Km value = 6.

Table 1 shows the MSG dose (mg/Kg bodyweight) fed to the respective rat groups (I–IV) as well as

the calculated HED (mg/Kg bodyweight) for the equivalent of a 9-year-old child.

Table 1 The MSG dose (mg/kg bodyweight) fed to groups I–IV and the HED calculated for the equivalent of a 9-year-old child.

GROUP	RAT DOSE (mg/kg)	HED (mg/kg)	NO. OF ANIMALS
I	2000	480	3
II	4000	960	3
III	20,000	4800	3
IV	30,000	7200	3

Euthanasia and organ fixation

The animals were euthanized using chloroform at the end of the fifth week after overnight fasting.

Histopathological analysis

After euthanasia, the tissues (colon, stomach, liver, kidney and brain) were immediately removed and washed with ice-cold normal saline solution, and then fixed in 10% formalin for a minimum of 24 hours. After embedding the tissues in paraffin wax, thin sections of 5 μm thickness were made using a rotary microtome. The sections were then stained with Hematoxylin and Eosin. The histopathological slides were examined and photographs were taken using a Zeiss Axio Skop 40 photomicroscope at 40x magnification.

Statistical analysis

To determine the statistical significance (p-value) of the body weight analyses, the control group and each independent MSG group (I–IV) were compared using the student's t-test in Excel.

RESULTS AND DISCUSSIONS

Mortality

There was no evident mortality until the end of the study, except in group IV. Group IV, fed with the highest dose 30,000 mg/kg, exhibited mortality as at the end of experiment only two animals were left out of three. Group IV animals also showed development

of polyps in their intestines which were evident upon histological analysis (Figure 1).

Body weight analysis

The weight of the rats were measured once per week and the results are shown Figure 2. Figure 2 shows that there was a constant increase in the body weight in all the groups. Statistically significance ($p\text{-value} < 0.05$) using Student's t-test was obtained by comparing respective MSG group with the control group. Increased body weight was observed in all the MSG treated animals with statistical significance ($p\text{-value} < 0.05$) except

group I where the $p\text{-value}$ was $0.09 > 0.05$. However, all the rats increased their body weights.

MSG (4-10 doses) administered subcutaneously or intraperitoneally to the neonatal rats induced visceral obesity and in particular, it increased the deposits of subcutaneous, gonadal and visceral adipose tissue, Lee index and body mass index (20). A previous study reported that when the young male Sprague–Dawley rats were allowed to consume MSG spontaneously, they exhibited reduced weight gain compared to the control (21). However, the present study show that there was a constant weight gain in male Wistar albino

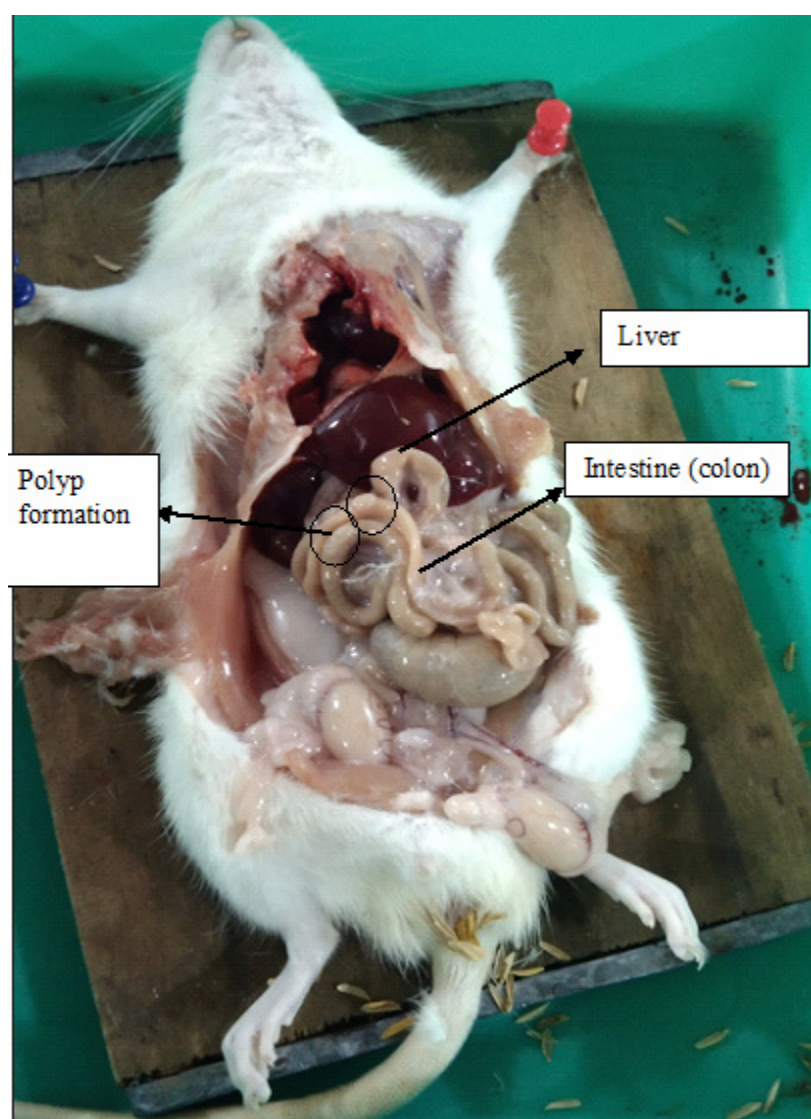
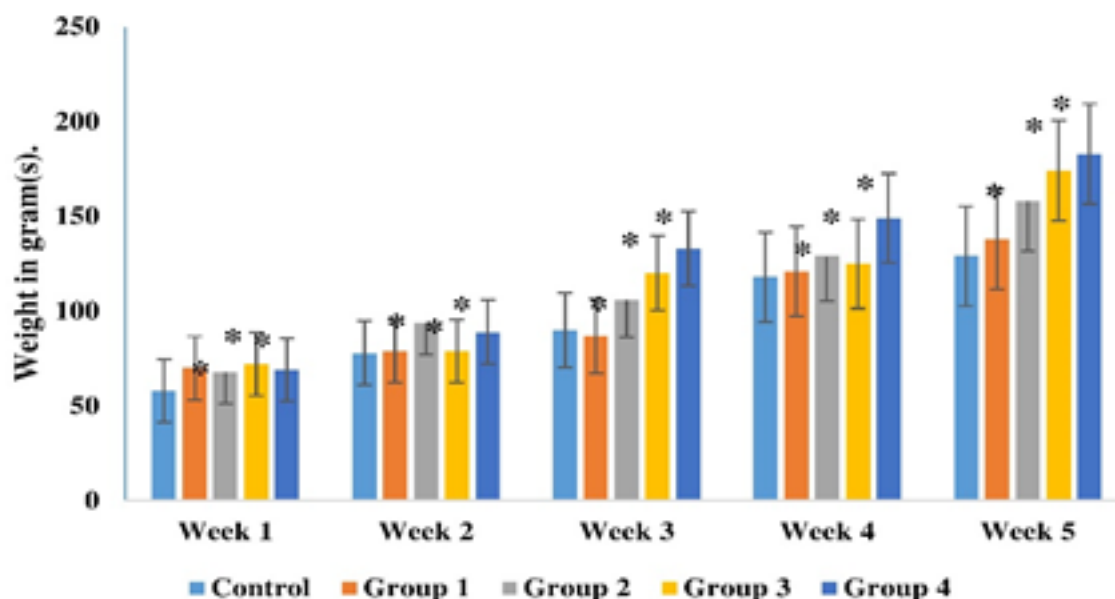


Figure 1 Image of the MSG group IV experimental Wistar albino rat showing the formation of polyps (marked as black circles) in the colon.



* Statistical significance p-value <0.05.

Figure 2 The graphical representation of weekly body weight change in the control and MSG fed groups. The graph shows that there is a constant increase in the body weight in all the groups.

rats fed MSG among Groups I, II, III, IV similar to the control group, particularly the Group IV animals with higher dose 30,000mg/Kg body weight show drastic increase in body weight in week five. This may be as a result of the high dose of MSG similar to those reported by Araujo *et al.*, (2017) who showed that “In adult mice, MSG consumption causing obesity due to the disturbed leptin-mediated hypothalamus signalling pathway”(22). The study showed an increase in body weight making the assumption it had been caused by the higher amount of MSG and it also correlated with the previous reports stating MSG causes obesity.

In this study, as the sample size was low, and the duration of MSG consumption was only five weeks, the body weight data analysis could not clearly define the role of MSG in body weight analysis. However, it could be said that initial consumption led to increase in body weight but damages to the organs caused by prolonged MSG consumption might lead to a decrease in the body weight.

Histopathology of the colon and rectum

Digested food is stored as faecal material in the colon which is connected to the rectum. The histology of the colon and rectum is important because the MSG adsorption occurs in the colonic mucosa. The colon cell structure consists of epithelium, muscularis mucosa and sub mucosal layers and has round shaped goblet cells, lamina propria, undifferentiated cells and endocrine cells were present at the base of the colonic crypts; paneth cells were usually absent. Whereas rectum cells were observed with simple columnar epithelium to keratinized squamous epithelial layer, the crypts become shorter and shorter in the rectum nearer to the anus region, and they were also observed with tattered and disappearing muscularis mucosa. Figure 3 shows the histopathological comparison in H and E stain of the colon and rectum of control *versus* rats fed MSG.

In this study the histology results from colon tissue

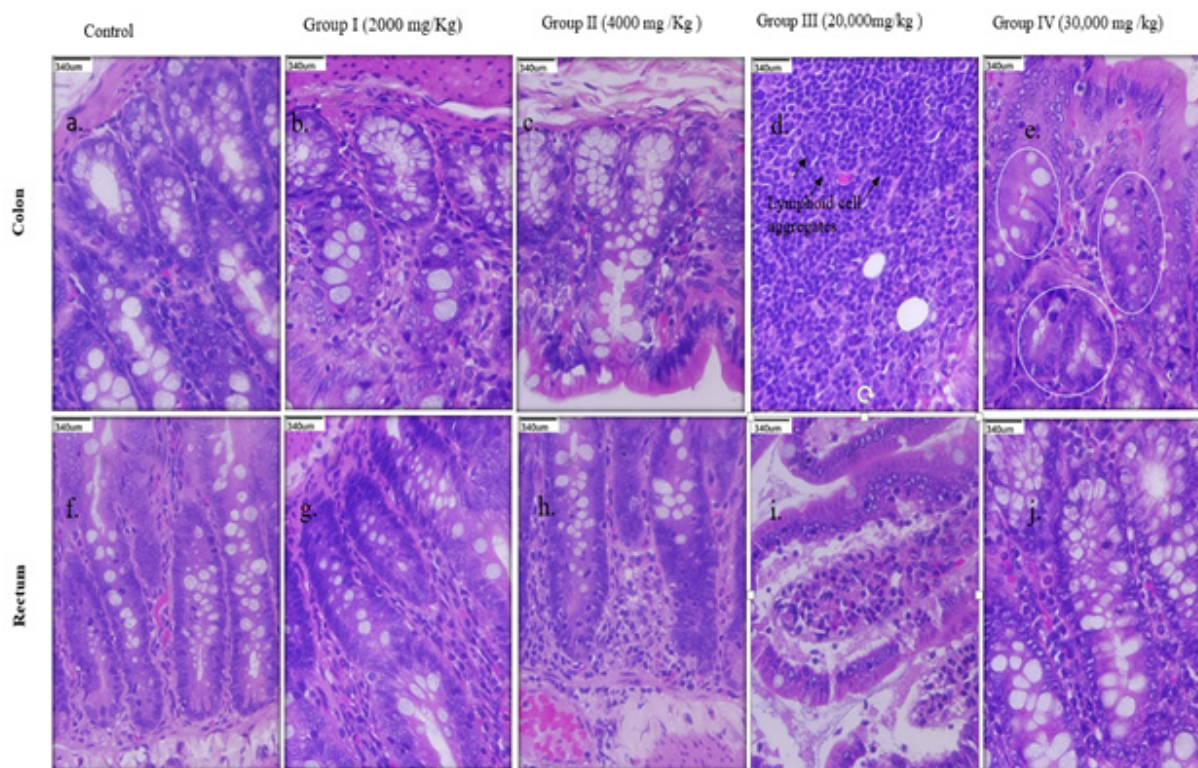


Figure 3 Histopathological images of the colon (a – e) and rectum (f – j). a= control colon; b=2,000 mg/Kg b.w MSG treated colon of group I rats; c= 4,000 mg/Kg b.w MSG treated colon of group II rats; d= 20,000 g/kg b.w MSG treated colon of group III; e= 30,000 g/kg b.w MSG treated colon of group IV animal. The histopathology of colon crypts results revealed that the rats in Group IV were observed with focal aberrant crypt hyperplasia (e) indicated in white circles compared to control (a); Group III colon noted with focal lymphoid aggregates (d) were indicated by the arrows. However, the colonic mucosa observed with preserved architecture.

showed that in Group IV, the formation of aberrant crypt foci (ACF) occurred (indicated by white circles in Figure 3), and Group III with numerous lymphoid cell aggregates. However, the colonic tissue in Group I and Group II appeared to normal, similar to the control. The well-known biomarker aberrant crypt foci (ACF), which are produced as a preventative response to food items and chemicals, are seen in colon cells and indicate a risk for colorectal cancer (23). In CRC the formation of ACF are reported as the early neoplastic lesions (24). Altered genes result in deregulated cell cycles, differential cell division and proliferation that ultimately leading to progression from adenoma cell to carcinoma cells (25) which is the outcome of a *de novo* malignant pathway of colonic mucosal cells. More than 90% of colon cancers arise as adenocarcinomas from epithelial cells of the colorectal mucosa (26).

In this study, Group IV rats were fed a high dose of MSG (30,00mg/Kg body weight) and exhibited ACF formation which was similar to the results obtained by Hata *et al.*, that is the number of ACF formations did not show any differences between the saline azoxymethane treated mice with MSG treated mice (i.e., MSG-AOM mice ACF formations = saline – AOM ACF formations) which means that tumorigenesis process in both group of mice were more or less similar (7). According to Hata *et al.*, 2012 the MSG-AOM mice are highly susceptible to colorectal carcinogenesis (7). The findings also indicate that the intake of high amounts of MSG could cause colon cancer when the level goes beyond the recommended dose. This study used one month-old rats equivalent to children who are more likely to have higher intake of MSG.

In the Group II rats, lymphoid cell aggregates were observed in the colon which might be due to inflammation, but Groups I, and IV showed no active inflammation. However, the colon mucosae were with well-preserved architecture in all the groups of MSG-fed rats compared to the control rats. Also, the histopathology results of the colon were similar to the results obtained by Eweka AO and Om'Iniabo (2007) in adult Wistar rats (27). The microanatomy of the small intestine showed toxic effects due to MSG consumptions at higher doses (3 g and 6 g of MSG mixed thoroughly with the feed, and given daily for fifteen days (27, 28).

The histopathology of the liver

The liver is an important hub for metabolic processes as it detoxifies chemicals, acts as reservoir of blood, secretes bile and cholesterol, and is the site of glucose metabolism, etc. Liver cells are usually designated as hepatocytes. The liver is organised as polygonal prism shaped structures called lobules which contain the

hepatocytes organised by hepatic cords and sinusoids. The blood flows through the hepatic artery carrying oxygenated blood from the aorta, whereas blood from the intestine and spleen passes through the hepatic portal vein. Blood from the intestine, spleen and aorta mixes at sites called sinusoids. Compared to the control, the treated animal liver, damage such as congested portal vein and degeneration of hepatocytes was observed in all the livers of the MSG fed rats. Histopathology of the MSG treated groups exhibited mild focal portal congestion (b-e) and mild feathery degeneration (f-i) which are marked by arrows in Figure 4. The livers of the control groups appeared normal. The results were analogous to that of the deleterious effect of MSG consumptions at higher doses (0.04 g/kg and 0.08 g/kg per day) in adult Wistar rats' livers and the malfunctioning of the liver (29). Recent research by Elbassuoni *et al.*, (2018) on the protective effect of L-arginine and Vitamin D against MSG-induced liver and kidney dysfunctions reported that “the animals given MSG alone noticed with increased body weight and had liver and kidney

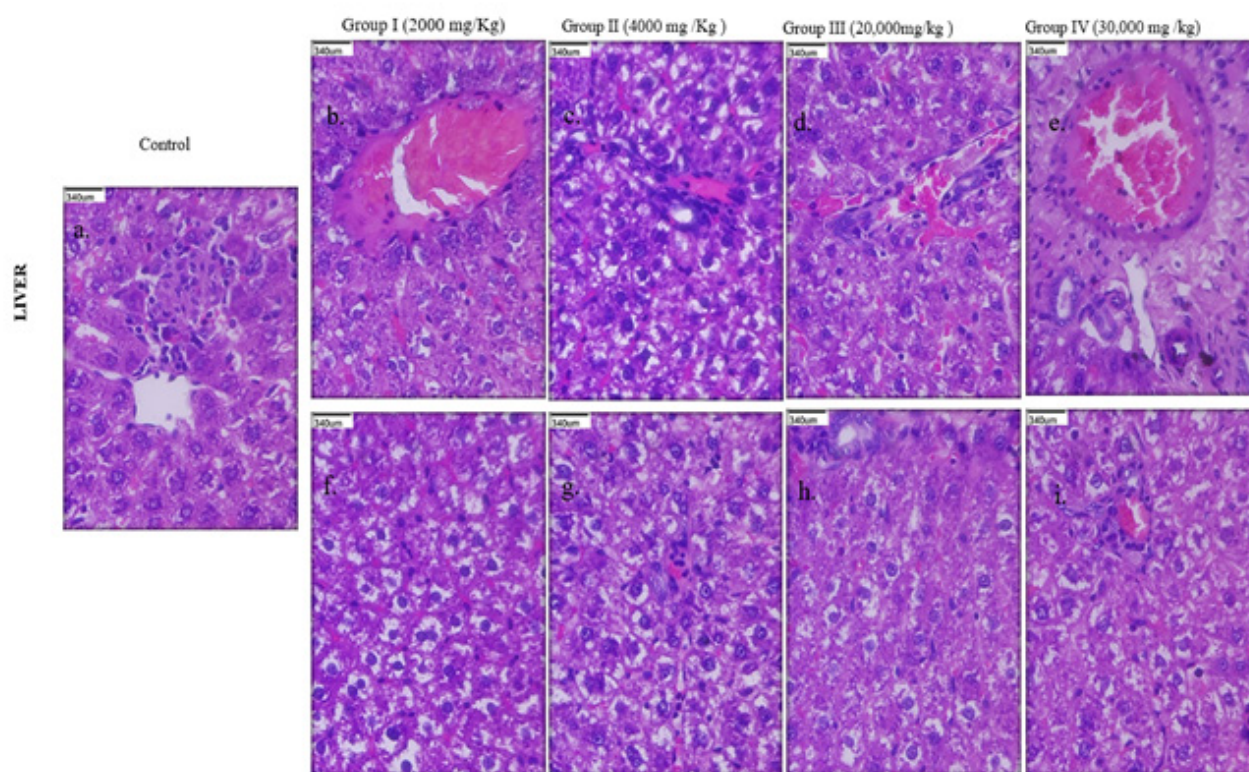


Figure 4 Liver parenchyma with preserved architecture. However mild focal portal congestion were seen (b-e) and mild feathery degeneration were noted and marked by arrows (f-i).

dysfunctions and also MSG was proved to induce oxidative liver and kidney damages” (30). In another very recent toxicology study, it was reported that MSG (dose of 30 g/kg feed) caused hepatotoxicity in adult Wistar albino rats and stated that *Nigella sativa* seeds had defensive role against the hepatotoxicity caused by MSG (31).

The histopathology of the kidneys

The kidney plays a major role in homeostatic functions. The formation of urine by filtering the blood impurities, via glomerular filtration and tubular reabsorption, is the main function. Renal corpuscles are the histologically important structures for the filtration process. This unique renal-corpuscles-filtration-barrier contains three crucial histological structures namely glomeruli (capillary endothelium), specialized cells called podocytes, and the fused basement membranes of both glomeruli and podocytes. The histology of kidney cells the in the control group and MSG fed rats are shown in Figure 5. Even though the results of kidney histology did not show any major dysfunctions, congested focal blood vessels were observed in all the MSG fed rats. However, renal parenchyma showed glomeruli with no significant pathological findings (a-e) and tubules appeared normal (a-e), with no evidence of interstitial

inflammation or fibrosis Figure 5. The present study kidney histopathology results show that there is a mild congestion of the glomerular tuft, but still there are no significant notable changes in the renal structure, thus the study results disagree with the postulation that “the effect of MSG on the cortex and medulla of kidney was degenerative” (32). However, the study agrees with the results on MSG obtained by Al-Sharkawy *et al.*, (2017); the kidneys showed mild congestion of the renal blood vessels (33). Recent research demonstrated, in male Wistar rats normotensive and hypertensive models, that daily consumption of MSG (320 mg/kg BW) for 8 weeks induced oxidative stress, significantly decreased the renal excretion of salt and water in both sham operation (SO) and 2-kidneys-1-clip (2K1C)(34).

The histopathology of the stomach

The stomach is located between oesophagus and duodenum and is a crucial part of the gastrointestinal tract. The stomach wall is divided into four distinctive layers called serosa, muscularis externa, sub mucosa and mucosa. The stomach histology results of the MSG fed rats groups were similar to the control group, and there were no pathological observations noted in the stomach. Figure 6 shows the histology of stomach.

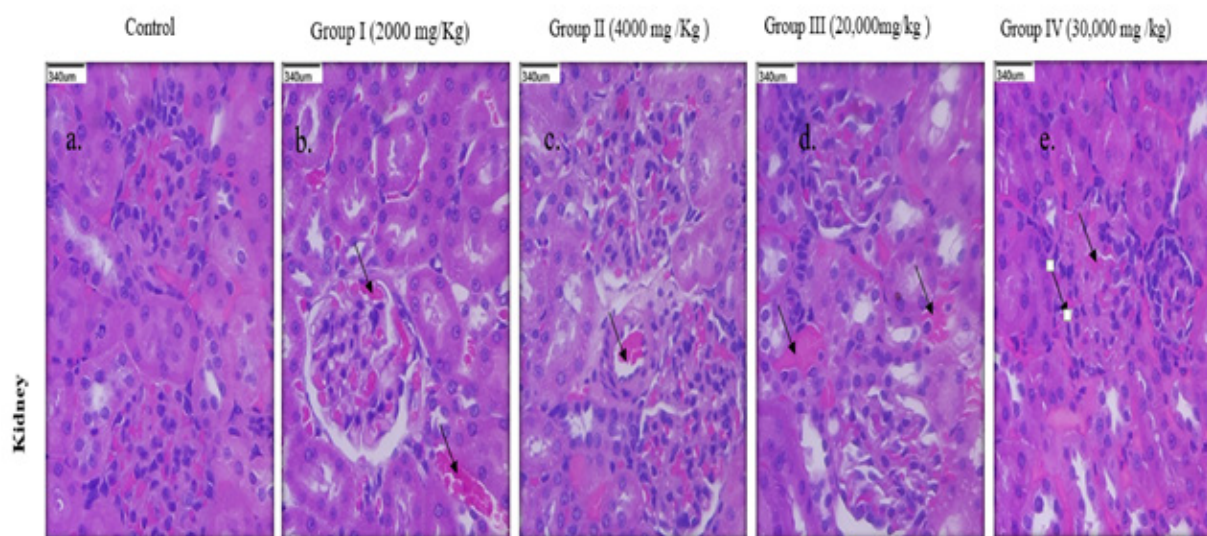


Figure 5 Renal parenchyma showing glomerular with no significant pathological findings (a-e). Tubules appear normal (a-e). No evidence of interstitial inflammation or fibrosis. Blood vessels shows focal congestion (b-e).

The histopathology of the brain

Neurons and glia are the parenchymal cells present in brain. Any damages to the central nervous system result in proliferation of glial cells or hypertrophy of different types of glial cells like astrocytes, microglial cells and oligodendrocytes. Necrosis is the process which refers to the loss of neurons and glial cell in the brain. Neuronal aggregates are formed due to the defect in the proteostasis mechanism of neurons. Figure 7 shows the histology of the brain tissues. Focal gliotic changes, neuronal necrosis, congested blood vessels and formation of neuronal aggregates were observed. At 10x magnification the brains of the control group shows no significant pathological findings and no evidence of reactive gliosis in the necrosis neural parenchyma. Group I (2000 mg/Kg body weight) tissues showed focal gliotic changes and congested blood vessels. Group II (4000 mg/kg body weight) had only congested blood vessels and Group III (20,000mg/Kg body weight) showed neuronal aggregates (marked in the red box) and congested blood vessel. Group IV (30,000mg/Kg body weight) showed focal gliotic changes indicated with orange arrows and congested blood vessels (black arrows). In magnification 40x: the control appears normal. Groups I, II & III show congested blood vessels indicated with black arrows. Group IV with

red arrows shows ballooning degeneration of neurons. Brain tissue histopathology revealed that all the four MSG treated groups showed congested blood vessels, focal gliotic changes were observed in Group I and Group IV, whereas Group III showed the aggregation of neurons. The results obtained are more relevant to the previous interpretation by histological study stating that consumption of MSG orally at acceptable daily doses clearly promoted injuries such as gliosis, blood capillary collapse, occluded blood vessel and so on in the brains of infants (35), and our results were analogous to the reports revealing the excitotoxicity of MSG. Altering of the structure and function also caused neuronal damages in auditory system and brain stem of SD rats (36). It was reported that MSG was found to increase the formation of free radicals, cause damage to the DNA and induce apoptosis (cell death) signaling leading to neural cell necrosis (37). Ahanger *et al* 2021 studied the role of MSG in protein aggregation due to protein misfolding by using kinetic measurements (38). The current study revealed that malfunctioning/changes in brain such as congested blood vessels, gliosis, degenerated neurons and formation of neuronal aggregates in MSG treated groups suggests that MSG consumption collapses the brain's cell structure. More research is needed to understand the molecular mechanisms.

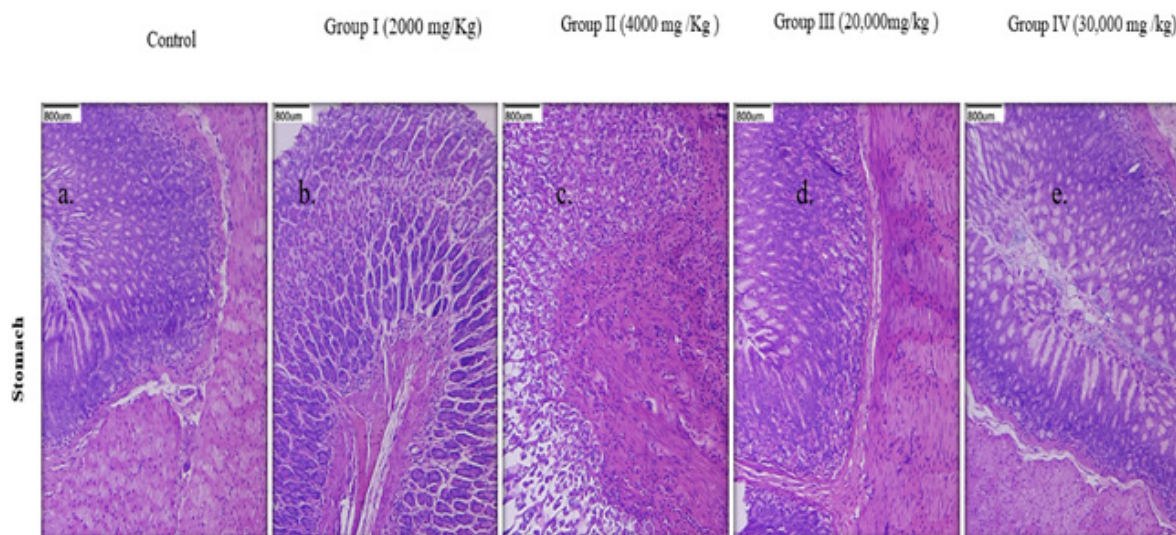


Figure 6 Gastric mucosa with preserved architecture and no evidence of chronicity changes or activity were seen (a-e).

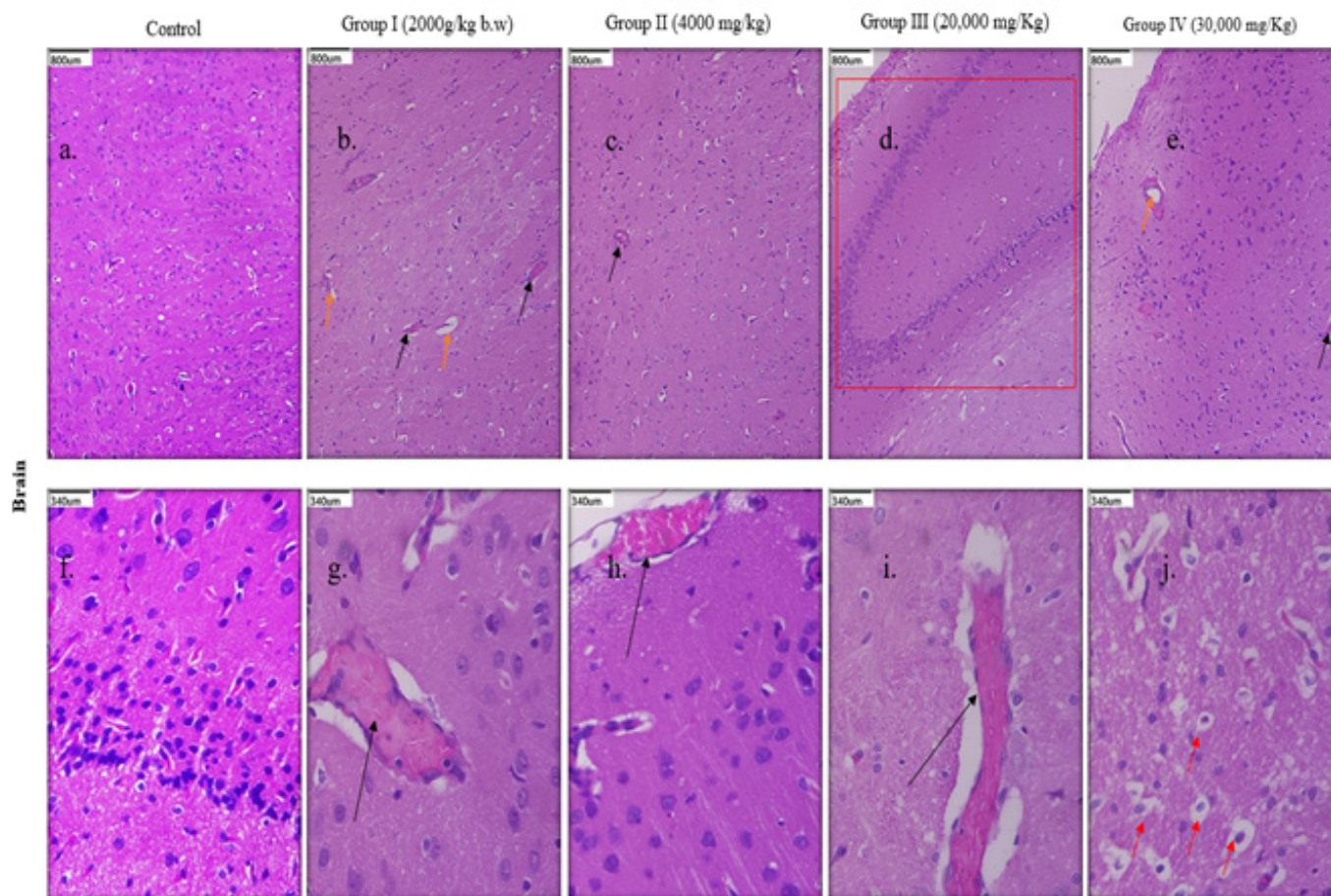


Figure 7 10x magnification of the control brain's neural parenchyma shows no significant pathological findings and no evidence of reactive gliosis, necrosis (a). Group I (2000 mg/Kg b.w) shows focal gliotic changes and congested blood vessels (b); Group II (4000 mg/kg b.w) had only congested blood vessels (c); Group III (20,000 mg/Kg b.w) showed neuronal aggregates (marked in red box) and congested blood vessels (d); Group IV (30,000 mg/Kg b.w) showed focal gliotic changes indicated with orange arrows and congested blood vessels (black arrows) (e). 40x magnification shows the control group to appear normal (f); images from g to i black arrows indicating the congested blood vessels; however group IV red arrows shows ballooning degeneration of neurons (j).

CONCLUSIONS AND FUTURE PERSPECTIVES

This study shows that the consumption of MSG by one month-old Wistar albino rats (equivalent to a 9-year-old child) resulted in noticeable physical changes in the rats. The results confirm the deleterious impact of MSG consumption when overfed in one month-old Wistar albino rat's organ such as colon, liver, kidney and brain. This study suggests that higher consumptions of MSG in children might affect their health adversely. Hence it is wise to avoid using the MSG as food additive in children.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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