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Monosodium Glutamate Level in Kid's Food and Its Dietary Effects on Liver and Kidney Functions in Adult Rats

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Abstract Youngsters are inclined to pleasing flavor and express a preference for sweetly, salty, and tasty food items than others. That's why monosodium glutamate (MSG) or tasting salt is using enormously in kid's food items to increase palatability known as umami taste though it has adverse impacts on health. The purposes of this study were to determine of MSG level in kid's items such as chips and noodles spices that are randomly taken by them and also its dietary consequences on liver and kidney functions in albino adult rats. The quantitative analysis was done by using the UHPLC system and 36 adult albino rats were used in this study for dietary intervention. The results showed that the overall mean value of MSG in imported chips was about doubled than the local brand. The MSG levels of the noodles spice varied from local to import and brand to brand which extended from 3.87±0.21-7.33±1.62 g/kg. Bodyweight of rats was significantly raised after the oral intervention of about 45.68%, 56.19% substantial in treatment 1(0.5 mg/g body weight of MSG) and treatment 2(1.5 mg/g body weight of MSG) respectively confronted with of about 22.59% in the control group. On regard to liver functions, the level of serum Alanine aminotransferase (ALT) was significantly ($p < 0.05$) increased but on the contrary, Albumin and Bilirubin were decreased with an uplift of MSG in treated rats compared to the control. MSG had antagonistic impacts on kidney functions as serum creatinine was significantly increased (35.48, 77.42%) inT1 and T2 group rats and serum urea was also increased in treated animals contrasted with the control group. The results unveiled that MSG at a low dose may causes an adverse outcomes on the hepatic and renal functions.

Keywords: *monosodium glutamate, kidney, liver, alanine aminotransferase, creatinine*

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1. Introduction

Monosodium glutamate (MSG) is a food additive, which serves as a useful flavor and taste enhancer. Its use has increased in recent times in Bangladesh as a flavoring in cooking to increase palatability and mostly in kid's food [1]. It is the sodium salt of glutamic acid, which is one of the most abundant amino acids in human foods. MSG, not only plays a part in taste enhance but also in developing the color of food items, like other amino acids to undergo Maillard type reactions in the presence of reducing sugars. MSG has a role to improve a unique taste called umami as it has chemical properties that active the taste buds of the tongue. However, only 0.2 - 0.8% of MSG by weighed can be the ideal palatability concentration and its use tends to be self-limiting as over-use reducing palatability. The largest palatable dose for humans is about 60 mg/kg body weight [2]. But in recent time most of the food producers use a higher amount of MSG in their food items especially to baby foods than the ideal level to make it more palatable. Consumption of MSG above the allowed limit has been associated with neurotoxic effects resulting in brain cell damage, retinal degeneration, endocrine disorder and pathological conditions such as addiction; stroke, epilepsy and it also have adverse effects on liver and functions [3].

The liver is the most important organ in the human body which has crucial metabolic processes functions such as detoxification, deamination, transamination, elimination of ammonia in the form of urea, biosynthesis, and release of the non-essential amino acids and plasma proteins with the exception of immune gamma globulins, gluconeogenesis, storage of glycogen, conversion of carbohydrates and proteins into lipids, synthesis of lipoproteins, phospholipids and cholesterol, oxidation of fatty acids, storage of iron in the form of ferritin as well as storage of vitamins A, D, and B12. Several enzymatic functional tests have been formulated to explore hepatic statuses such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, some other

tests include measurement of serum lactic dehydrogenase (LDH), albumin, bilirubin, gamma-glutamyl transpeptidase (GGT), alkaline phosphatases and 5-nucleotidase activities are employed [4]. The kidneys are another two bean-shaped vital organs in the renal system located in the posterior abdominal wall whose major roles include the discharge of toxic metabolites and waste products from the blood and control of the volume of fluid and electrolytes equilibrium in the body. To test functions of the kidneys routine urinalysis is used to measure serum urea, creatinine, sodium and potassium and serum bicarbonate [5].

In recent years Bangladesh is experiencing an increasing trend of Non-communicable diseases (NCD) due to changing dietary patterns and sedentary lifestyles. The World Health Organization (WHO) has been documented in 2017 stating that 2.84% and 1.94% of total deaths in Bangladesh are due to liver diseases and kidney disease respectively [6]. These types of death prevalence are rising day-by-day as most of the people are affectionate of taking their meal from the restaurant due to their busy schedule or use to make savory and ready to eat items and the parents also feed their Children that are ready to eat or ready to cook. The most two common foods which are liked by kids are chips and noodles which they have taken as school tiffin or afternoon snacks regularly. That's why the objectives of this research were designed to determine monosodium glutamate levels in baby food items such as chips and noodle's spices and also study the effects of MSG on liver and kidney functions in albino rats.

2. Materials and Methods

2.1. Quantitative Analysis

The quantitative examination designed that involves sampling, sample preparation, and analysis using a blank as a control to determine the levels of MSG in samples by the UHPLC machine. Quantification was done using standard curves and significant levels between brands using analysis of variance.

2.1.1. Sampling and Sample Preparation

Samples were bought from the local market and super shop of Chattogram city corporation, Bangladesh. Cluster, random and an arbitrary sampling method used by the following formula $n = 0.5\sqrt{N}$, where n is the sample size and N is the total population [7]. A total number of 14 chips (7 local and 7 imported) and 10 noodles (5 local and 5 imported) brand samples were taken according to formula. Samples were then brought to the laboratory, labeled and refrigerated at 4°C until preparation and analysis. After that Samples were mingled thoroughly by blender and homogenized by shaking vigorously.

2.1.2. Stock Solution Preparation

A stock solution of 5 mg/ml of each sample was prepared by dissolving 0.5 g of the sample into 100 ml of double-distilled deionized water. The pH of the solution was adjusted to 7.7 using 5 % w/v NaHCO3. The solution was then filtered using Whatman filter paper No.1. A 0.5 ml of filtered sample solution was taken and put into Eppendorf tube and pre-column derivatization done using dinitrofluorobenzene (DNFB) [8].

2.1.4. Standard Curve Establishment

The standard curve of derivatized standard MSG of 0,5, 10, 20, 30 and 40 µg/ µl was plotted to determine the quantity of MSG in food samples. The peak of MSG was identified by comparing it with the retention time of MSG standards, which was 6.8 minutes. The peak area was used for the quantitative calculation of MSG.

2.1.5. UHPLC Analysis

Agilent 1100 (Agilent Technologies, USA) machine was used to measure the formaldehyde where the column was Hypersil ODS2-C18, DAD Lamp mode: D2, UV detector set before running the samples. The syringe volume of the autosampler was 100µl and the autosampler and column oven temperature was at 20°C and 30°C respectively. The sample volume and the absorption wavelength of the detector were set at 20µl and 254 nm apiece. The Mobile phase has consisted of methanol: water (1:1) v/v) with a flow rate of 1.2 ml/min.

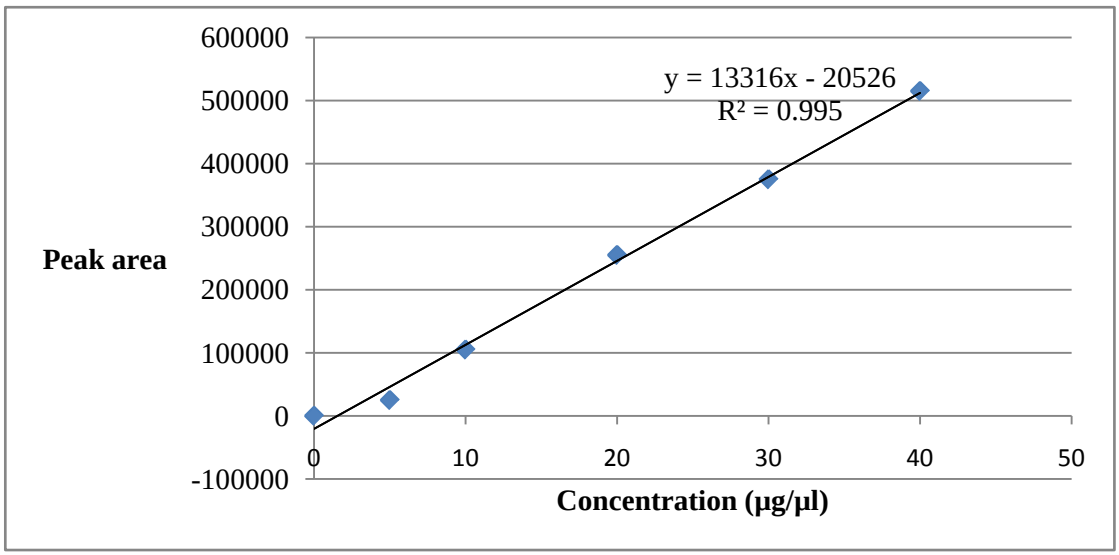

Figure 1. Calibration curve for monosodium glutamate

2.2. Experiment on Animals

2.2.1. Animals Collection

Adult albino rats of both sexes with an average weight of 95 g were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram. The animals were housed in cages under a standard hygienic condition and were fed by using oral gavage feeding tubes and water by labium. To optimize treatment doses, all animals have fasted for 1 hour before treatment administration.

2.2.2. Experimental Design

A total of 36 animals were adopted in this experiment. The animals were weighed and randomly distributed into 9 cages (each cage contained 4 animals of approximate equal body weight). The cages were then randomly allocated to three experimental diets with 3 replicates (12 animals per treatment) for 30 days. The experimental diets were approximately similar in all other aspects except in the two levels of MSG 0.5 and 1.5 mg /g body weight of T1 and T2 group respectively, the control group was in normal diet except MSG.

2.2.3. Blood Collection

At the end of the experiment, rats were sacrificed 24 hours following the last given dose and serum was separated from blood by centrifugation at 3000 rpm for 10 min and stored at -80°C bending biochemical analysis.

2.2.4. Biochemical Assays

All blood serum were evaluated by Fully Automatic Biochemistry Analyzer FACA-261(Labomed, Inc, USA). Summary of test principle and clinical relevance-

2.2.4.1. Assessment of Liver Function

Some blood tests used to help diagnose and monitor liver disease or damage. The examinations measure the levels of several enzymes and proteins in the blood. In this study, we analyzed three parameters such as Alanine Aminotransferase (ALT), Albumin and Bilirubin level in blood serum as an indicator of wellness of the liver.

ALT is an enzyme found in the liver that is an indicator of the wellness of the liver if it increased in the bloodstream that means liver damaged. Serum elevations of ALT activity are rarely observed except in parenchymal liver disease since ALT is a more liver-specific enzyme than aspartate aminotransferase (AST).

Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys. Albumin is one of several proteins made in the liver which is used to fight infections and to perform other functions. When the bloodstream contained a lower amount of albumin represent liver damage or diseases.

Bilirubin level in serum also expresses the liver dysfunction is a substance produced during the normal breakdown of red blood cells. Bilirubin passes through the liver and is excreted in the stool. Elevated levels of bilirubin indicate liver damage or disease or certain types of anemia.

2.2.4.2. Assessment of Kidney Function

Metabolic wastes, extra fluids, and foreign bodies are removed from the body through both kidneys. There are some bloods and urine examinations done in the laboratory to know how well the kidneys are doing their job and how quickly body wastes are being removed. In this study, we have done serum creatinine and urea test for knowing the effects of MSG on kidney functions.

Serum creatinine generally is the first test used to assess kidney function, is a waste product that comes from the breakdown of protein in the food increased if the kidney function decreased

2.3. Statistical Analysis

Outcomes obtained from the experiment were analyzed using analysis of variance (ANOVA). Tukey's test was used to observe the multiple comparisons. However, in case of violation of homogeneity comparisons were made using the Games Howell's test at $P< 0.05$ level of significance.

3. Results and Discussion

The monosodium glutamate levels were measured in Chips and Noodles spices collected from the supermarket of Chattogram, Bangladesh. Each sample was analyzed three times separately using the UHPLC system. The results revealed that imported chips (5.2 ± 0.96 g/kg) contained a significantly higher amount of average value of MSG than local brands (3.0 ± 0.5 g/kg) are presented in Figure 2. Conversely, in the case of noodles spices, the average value of MSG for imported brands (4.69 ± 1.08 g/kg) was remarkably lower than the local brands (6.8 ± 0.35 g/kg). Furthermore, within brands failed to reach the statistically significant difference value for noodles spices but in chips, within brands both imported and local, the MSG content significantly differed from each other.

The results secured are comparable to a study of free and natural glutamate levels in a variety of foods in which the MSG range was between 2.3 - 12.7 g/kg [9] and other analyses of Pakistan spice brands where the ranged between 27.0 - 88.0 g/kg [8]. Comparing the mean levels of MSG with what has been accepted as the maximum allowed limit (10 g/kg), in European countries [10]. However, the mean level of MSG in both samples was lower than the allowed limit by European countries.

Association of the final body weight with initial in each group showed a significant higher and hence the increase of about 45.65% and 56.19% were noteworthy in T1 and T2 respectively compared with an increase of about 22.59% in the control group (Figure 3). MSG intake could provoke an increase in energy intake as it acts as a taste enhancer which could lead to obesity in T2 > T1 group rats. This result is similar to a study where they found an increase of about 34.6%, 44.5% in weight after ingestion of 0.6 and 1.6 g MSG/ kg body weight respectively [4]. In this study, the weight gain was slightly higher than the previous study; this may be due to the different feed grains, weather, rat species and, the season of the rearing.

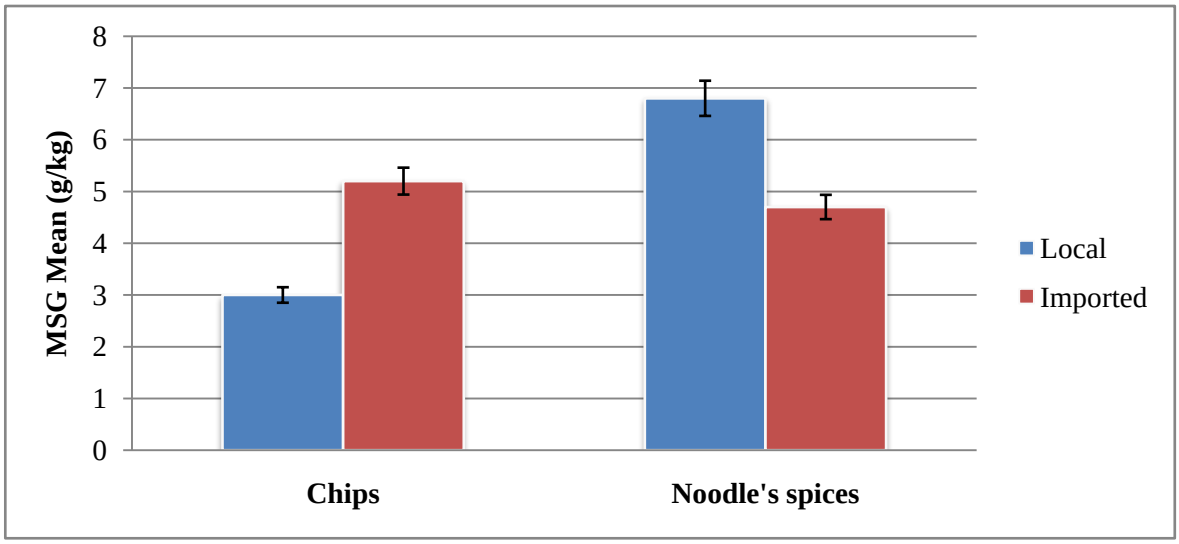

Figure 2. Comparison of average MSG level in local and imported chips and noodles

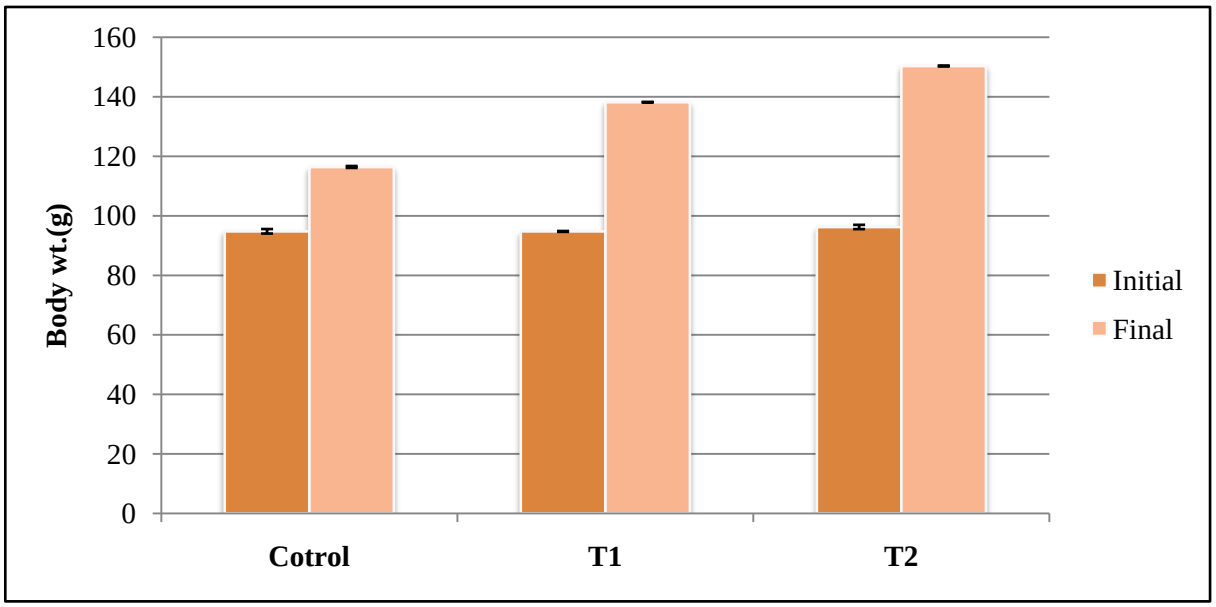

Figure 3. Changes in body weights of control and rats treated with MSG

Table 1. Liver Function Indexes of the Control and Treated Rats

Group	ALT(U/L)	Albumin(g/L)	Bilirubin(mg/dl)
Control	32.03±0.43^{a}	6.20±0.26^{a}	0.61±0.01^{a}
T1	40.03±1.34^{b}	5.73±0.56^{a}	0.59±0.03^{ab}
T2	45.53±0.53^{c}	4.90±0.29^{b}	0.52±0.05^{b}

Note: Data are presented as Means ± SD and within the column bearing different superscripts are significantly different (P< 0.05).

Table 1 shows the liver function indices such as serum Alanine aminotransferase (ALT) significantly ($p < 0.05$) increases but on the contrary, Albumin and Bilirubin were decreased with the higher amount of MSG-treated rats compared to the control rats. From the results, ALT had activities means of 32.03±0.43, 40.03±1.34 and 45.53±0.53 U/L in the serum of the control and MSG-administrated adult rats T1 and T2 respectively. The increase in ALT value indicates the liver damage or disease as it is a sensitive marker of liver damage [11]. This increase of serum ALT could be free radical production induced by MSG which reacts with the liver cell membrane and damage the cellular structure, resulting in enzyme leakage [12]. The result of this study similar to the report of other studies where, serum ALT value elevated with the increase of MSG dose in rats [13,14].

The serum albumin was found a decreasing (16.20 and 20.97%) trend in treated T1 and T2 group rats respectively where, the mean serum albumin was of about 6.20 ± 0.26, 5.73 ± 0.56, and 4.90 ± 0.29 g/L in control, T1 and T1 group rats. Besides, T1 failed to reach and T2 reach a statistically significant level with the control group. The determined mean concentration of serum bilirubin was about 0.61 ± 0.01, 0.59 ± 0.03, and 0.52 ± 0.05 mg/dl in control, T1 and T2 group rats sequentially. Furthermore, 3.28 and 14.75% reduction in serum bilirubin was observed in MSG treated rats confronted with the control group (Table 1). These results seem similar to the findings of other studies and reveal that decreased serum albumin and bilirubin are the indications of liver damage as it's the main organ of the synthesis and excretion of these to the bloodstream and from the body [15].

Kidney function indices (serum creatinine and urea) values are shown in Table 2. From the result, it was perceived that serum creatinine was significantly increased (35.48, 77.42%) inT1 and T2 group rats contrasted with the control group whereas, it had a mean value of about 0.31±0.03 mg/dl. Similarly, the serum urea concentration was of around 35.03 ± 0.40, 35.63 ± 0.88, and 37.25 ± 1.23 mg/dl in control, T1 and T2 rats group respective. In addition to this, the serum urea significantly rose in the T2 group but the T1 group had no significantly differ than the control group. The significant increase in creatinine and urea content of the serum following the administration of MSG may be associated with kidney dysfunction. Dietary MSG exposure may harm the renal function which might be due to oxidative stress on the renal tissue and hampered the excretion waste materials from the body [16,17,18].

Table 2. Kidney Function indexes of the control and treated rats with MSG

Group	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)
Control	0.31 ± 0.03^{a}	35.03 ± 0.40^{a}
T1	0.42 ± 0.03^{b}	35.63 ± 0.88^{a}
T 2	0.55 ± 0.05^{c}	37.25 ± 1.23^{b}

Note: Data are presented as Means ± SD and within the column bearing different superscripts are significantly different ($P< 0.05$)

4. Conclusion

The results of the present study have shown that MSG is present in noodles spices and chips at low doses but which is capable of increasing the body weight and damage the liver and kidney functions. These changes appear in the liver and kidney probably because these organs are mainly responsible for detoxification and excretion of foreign bodies in the body.

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