

Effects Of Mono-Sodium Glutamate Administration On Metabolic Parameters, Hepatic And Renal Functions In Adult And Neonate Male Rats.

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Abstract

Background: Monosodium glutamate (MSG) is used as food additive enhancing flavor of food. It was linked to many metabolic alterations, obesity, inflammation and oxidative damage in different organs. We investigated the possible metabolic effects of MSG and its effect on liver and kidneys in adult and neonatal male rats. Materials and methods: The study was carried for 12 weeks on forty male rats divided into four groups: MSG Adult were given (120 mg/kg/day) Intraperitoneal (I.P), MSG Neonatal rats given MSG (120 mg/kg/day) I.P, control adult were given (120 mg/kg/day) saline I.P and control neonates given (120 mg/kg/day) I.P saline. Final body weight, weight gain, body mass index (BMI) and Lee index were measured. Fasting blood glucose, serum levels of insulin, HOMA-IR, leptin, final liver and kidney weights, liver and kidney function tests (serum AST, ALT, serum urea and creatinine), serum TNF- α , total antioxidant capacity (TAC) and Mean ABP was measured. Histopathological examination of liver and kidney tissues was done. MSG induced a significant increase in final body weight, weight gain, BMI and Lee index in MSG neonatal and MSG adult rats. Significant increase in blood glucose, serum levels of insulin, leptin and TNF- α with significant decrease in serum TAC, liver and kidney functions were impaired in both MSG treated groups with histopathological alterations. Conclusion: MSG induced obesity, metabolic alterations including hyperglycemia, hyperleptinemia, insulin resistance and decreased antioxidant defense. MSG had a toxic effect on liver and kidneys and should be taken cautiously in diet especially for infants and children.

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INTRODUCTION

Monosodium glutamate (MSG) is composed of sodium salt of the glutamic acid, it is used as a food additive applied to give flavor and improve the taste [1]. MSG acts on glutamate receptors found in most tissues, in nerve terminals, brain, spinal cord, kidney, liver, testes, lungs, spleen and heart [2].

Daily dietary composition of glutamate varies from one race to another, as daily oral consumption ranges from 0.5 mg/kg between Americans and over 3g/kg in Taiwanese meals [3]. Despite MSG's widespread use, it has continued to be followed by controversies regarding its safety on different body organs [4]. However, It was considered "Generally Recognized as Safe" (GRAS) status by regulatory agencies when it is used as food additive [5].

Plentiful intake of MSG in processed or natural foods was shown to induce several physiological changes such as Obesity, metabolic disturbances, hepatotoxicity, renal abnormalities and endocrine abnormalities [6]. It was suggested that MSG added to food cause damages in most parts of the brain that control other parts of the body, resulting in neuronal, metabolic and endocrinal alterations both in young and adult exposed humans [7]. In addition, MSG was linked to the induction of obesity, because of its ability to increase the palatability and food intake, besides the disruption of the hypothalamic signaling cascade of leptin action and enhancing adipocyte capacity to transport glucose, insulin resistance and increased glucose levels [8]. Since MSG is metabolized in the liver and excreted via the kidneys [9], it is important to examine its impact

on these two vital organs and the possible mechanisms underlying these effects. Concerns about the safety of usage of MSG in different foods in human adult and children are raised after its use as method for induction of obesity and other different metabolic alterations and organ damage in both neonates and adult ages [10].

Based on the previous introduction, the aim of the study was to evaluate the possible metabolic abnormalities that could develop as result of MSG administration in a dose close to the usual doses taken in diet and illustrate whether these changes could be affected by the age of animals at the time of administration either at the neonatal or at adulthood periods and to investigate MSG-induced structural and functional changes in both liver and kidneys.

1. Material and Methods

2.1 Animals

Adult (3 months old) and neonate (10 days old) male Wistar Albino rats were used in the study. Animals were housed in clean, appropriately ventilated cages (4 rats per cage) in the animal house of the Faculty of Medicine, Assiut University. Their quarters maintained a standard light-dark cycle, at room temperature, with access to food and tap water throughout the entire study period. The rats were left for a week to acclimatize before the experimental procedures began. The research procedures were in accordance with the 'Guidelines of Experiments on Animals' and were accepted by the Ethics Committee at the Faculty of Medicine, Assiut University, Egypt. The rats were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Approval no.#17200599#).

2.2. Materials: MSG was purchased from Loba Chemie, India as white crystals.

Experimental design

Forty male rats were randomly divided into 4 groups:

Group I: (Control-A): 10 rats aged 3 months old were given a vehicle (saline) intraperitoneal injection (I.P) for 3 months.

Group II: (MSG-A): 10 rats aged 3 months old were given MSG (120 mg/kg/day) [11, 12] I.P for 3 months.

Group III (Control-N): 10 rats aged 10 days old were given I.P injection of a vehicle (saline) for 3 months.

Group IV: (MSG-N): 10 rats aged 10 days old administered MSG (120 mg/kg/day) I.P for 3 months.

2.3. Anthropometrical measures

Body weight was measured at the beginning of the experiment (initial body weight), at regular two week intervals throughout the entire study period, and on the day of euthanasia (final body weight). Weight gain (WG) was calculated as Final weight-initial weight, weight gain percent (WG %) was measures as (final BW- initial BW)/ initial BW X 100 [13]. Body mass index (BMI) calculated as body weight (g) divided by nasoanal length (cm²) Lee index was calculated as the cube root of body weight (g) divided by the naso-anal length (cm). Rats were considered obese when the abdominal circumference $>14.9 \pm 2$ cm and the Lee index >0.3 [14].

2.4. Blood pressure measurement: Done by rat tail pressure meter (**Pan Lab Harvard model LE 5001**)

Blood and tissue sampling:

2.5. Blood sampling:

Fasting blood samples were obtained at the end of the experiment and centrifuged for 15 min at 3000 RPM. The clear supernatants (sera) were taken and distributed into small aliquots and kept at -20 °C until use.

2.6. Biochemical examinations:

2.5.1. Determination of fasting blood glucose levels from tail vein using an On-Call Ez glucometer (ACON Laboratories, Inc., USA).

2.6.2. Determination of serum insulin level.

Levels of serum insulin were determined using commercially available enzyme linked immunosorbent assay kits (ELISA) (CALBIOTECH, USA). All analyses were conducted according to the manufacturer's instructions

2.6.3. Calculation of Insulin resistance: HOMA-IR = (Fasting insulin in mIU/L x Fasting glucose in mg/dL) / 405 [15]. Normal reference levels for HOMA-IR range between 0.7 and 2.0.

2.6.4. Determination of serum leptin level

Levels of serum leptin were determined using commercially available ELISA kits (Biochem Canada inc. Canada). All analyses were conducted according to the manufacturer's instructions

2.6.5. Determination of serum Total antioxidant capacity (TAC)

Levels of serum TAC were determined using commercially available spectrophotometric kit purchased from Bio-diagnostics (Cairo, Egypt). All analyses were conducted according to the manufacturer's instructions

2.6.6. Determination of serum Tumor necrosis alpha (TNF- α .)

Levels of serum TNF- α were determined using commercially available ELISA Kit (Cusabio, USA). All analyses were conducted according to the manufacturer's instructions

2.6.7. Determination of serum Liver enzymes Aspartate transaminases (AST) and Alanine transaminases (ALT)

AST and ALT levels were determined using commercially available spectrophotometric kit purchased from Spectrum (Egypt). All analyses were conducted according to the manufacturer's instructions

2.6.8. Determination of serum Urea and creatinine Urea and creatinine levels were determined using commercially available spectrophotometric kit purchased from Spectrum (Egypt). All analyses were conducted according to the manufacturer's instructions.

2. Tissue sampling:

The liver and kidneys were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. Both organs were washed with cold saline solution and dried between two filter papers then weighed for final liver and kidney weights and calculation of liver and kidney index was done as follow: organ weight (g)/ BW(g)x100. After that, they were saved for the histopathological examination.

4. Histopathological Examination:

Specimens from the liver and kidney were taken immediately after weighed the organ of the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, and stained with Hematoxylin and Eosin (H&E) and Periodic acid

Schiff (PAS) stains and examined microscopically according to [16]. The Periodic acid Schiff (PAS) stain is used to highlight intrahepatic glycogen, which is stained dark pink. When the intrahepatic glycogen is markedly decreased, the cells are stained pale pink and the reaction is considered negative

5. Statistical analysis:

All data were expressed as mean \pm SEM. All data were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test as post hoc test. Results were analyzed by using the computer statistic Prism 3.0 package (Graphpad software, Inc, San Diego. CA, USA).

Standard curves were constructed for laboratory investigations and samples' content of the measured analytes was extracted from the standard curve by the same statistical analysis package. The minimum level of statistic significance was set at $P < 0.05$.

6. Result

6.1. Anthropometric results (Table1):

There were no significant differences in the initial body weight among the studied groups as compared to control groups of similar age. Final body weights, weight gain, BMI and Lee index were increased significantly in MSG-A and MSG-N as compared to control-A and control-N groups respectively indicating obesity in both MSG-A and MSG-N groups. In addition, there was a significant increase in WG, BMI and Lee index in MSG-N as compared to MSG-A group.

6.2. Fasting blood glucose levels, Serum insulin levels, HOMA-IR levels and Serum leptin levels (Table 2):

Fasting blood glucose levels were significantly increased in MSG-A as compared to control-A. It was significantly increased in MSG-N as compared to control-N group. There was no significant difference in fasting glucose levels between MSG-A and MSG-N. There was a significant increase in serum levels of insulin between MSG-A and control-A. Also, a significant increase in MSG-N as compared to control-N. There was no significant increase in MSG-N as compared to MSG-A.

Similar results were obtained in the HOMA-IR index as there was a significant increase in HOMA-IR in the MSG-A as compared to control-A and a significant increase in MSG-N as compared to control-N. There was no significant increase in HOMA-IR index in MSG-N as compared to MSG-A.

As regarding serum levels of leptin, there was a significant increase in serum leptin in MSG-A group as compared to control-A group and a significant increase in leptin levels in MSG-N as compared to control-N group. There was no significant difference in leptin levels between MSG-A and MSG-N groups

6.3. Results of serum TNF- α (Table 2):

There was a significant increase in serum TNF- α level in MSG-A as compared to control-A and a significant increase in serum TNF- α in MSG-N as compared to control-N. There was no significant difference in between MSG-A and MSG-N groups.

6.4. Results of serum TAC (Table 2)

As regarding serum TAC level, there was a significant decrease in serum TAC level in MSG-A as compared to control-A and a significant decrease in serum TAC in MSG-N as compared to control-N, while there was no significant

difference between serum TAC levels in MSG-A and MSG-N groups respectively.

6.5. Results of mean ABP (Figure 1):

Mean ABP was significantly increased in MSG-A group as compared to control-A group and in MSG-N group as compared to control-N group. There was no significant difference in mean ABP between control-A and control-N groups or between MSG-A and MSG-N groups respectively.

6.6. Liver final weight and liver index results (Table 3):

There was a significant increase in final liver weight in MSG-N as compared to control-N, while there was no significant difference in final liver weight in MSG-A as compared to control-A group or in MSG-A as compared to MSG-N.

Liver index was not significant among all study groups.

6.7. Results of final kidney weight and kidney index (table 3):

Final kidney weights were significantly increased in MSG-A group as compared to control-A group. There was a significant increase in final kidney weights in MSG-N group as compared to control-N group. There was no significant change in final kidney weights between MSG-A and MSG-N groups. The kidney index was found to be non significant in all four groups.

6.8. Results of liver enzymes (AST & ALT levels) (table 4)

Serum AST levels were significantly increased in MSG-A as compared to control-A groups. AST level also was significantly increased in MSG-N as compared to control-N. There was no significant difference in AST level between MSG-A and MSG-N groups.

Serum ALT was significantly increased in MSG-A as compared to control-A and it was significantly increased in MSG-N as compared to control-N. In addition, there was a significant increase in serum ALT in MSG-N as compared to MSG-A. .

6.9. Changes in serum urea and serum creatinine levels (table 4):

Table (1): Changes in initial and final BW, WG, Ratio of WG to initial BW, percent of WG, BMI, and Lee index among the study groups.

	Control-A	MSG-A	Control-N	MSG-N
Initial BW (gm)	101.9 ± 5.20	113.2 ± 2.25	47.56 ± 1.86	50.33 ± 1.83 ^{c***}
Final BW (gm)	212.3±6.73	265.7±8.91 ^{a***}	132.4± 8.41	255.0±11.55 ^{b***}
WG (gm)	110.40± 8.43	152.40± 7.25 ^{a**}	84.89± 5.79	208.30 ±11.06 ^{b***, c***}
BMI	0.39 ± 0.02	0.55 ± 0.03 ^{a***}	0.23 ± 0.20	0.74 ± 0.03 ^{b***, c***}
Lee index	0.26 ±0.01	0.30 ± 0.007 ^{a**}	0.21 ± 0.004	0.36 ± 0.01 ^{b***, c***}

Data were expressed as mean ± SEM. ^a Comparing MSG-A vs Control-A, ^b Comparing MSG-N vs. Control-N. ^c Comparing MSG-A vs. MSG-N. (* when p value <0.05, ** when p value <0.01, *** when p value <0.001).

Table (2): Changes in levels of fasting blood glucose, serum insulin, insulin resistance index (HOMA-IR), serum leptin, serum TNF- α and serum TAC among the study groups.

	Control-A	MSG-A	Control-N	MSG-N
Blood glucose levels (mg/dl)	71.89 ±2.16	120.8 ±5.4 ^{a***}	65.89 ± 2.35	124.7 ±4.9 ^{b***}
Serum insulin results (μIU/L)	5.74± 0.56	25.77 ± 0.56 ^{a***}	3.46 ± 0.49	31.25± 2.41 ^{b***}
HOMA-IR	1.00 ± 0.091	6.84 ± 0.4 ^{a**}	0.57± 0.09	8.66 ± 0.89 ^{b***}
Serum leptin (ng/ml)	63.27 ± 4.0	89.50 ±2.20 ^{a***}	66.35 ± 2.36	82.14 ±3.03 ^{b*}
Serum TNF-α (pg/ml)	23.20 ±1.58	46.20± 3.64 ^{a***}	22.13±0.84	52.13± 3.93 ^{b***}
Serum TAC (mM/L)	0.56±0.04	0.39±0.03 ^{a*}	0.45 ± 0.03	0.28 ± 0.02 ^{b**}

Data expressed as mean ± SEM. ^a Comparing MSG-A vs Control-A, ^b Comparing MSG-N vs. Control-N. (* when p value <0.05, ** when p value <0.01, *** when p value <0.001)

Table (3): Changes in final liver weight, Liver index, Final kidney weight and Kidney index among the study groups

	Control-A	MSG-A	Control-N	MSG-N
Final liver weight (gm)	6.11 ±0.36	7.07 ± 0.44	4.10 ± 0.32	5.75± 0.31 ^{b*}
Final liver weight/ final BW ratio(Liver index)	2.86 ±0.22	2.58 ± 0.15	3.08 ± 0.28	2.34± 0.21
Final kidney weight (gm)	1.62 ± 0.15	2.27± 0.21 ^{a*}	1.06 ± 0.11	1.71 ± 0.12 ^{b*}
Final kidney weight/final BW ratio (Kidney index)	0.77±0.07	0.86±0.09	0.83±0.08	0.72±0.08

Data shown are mean ± SEM. ^a Comparing MSG-A vs. Control-A, ^b Comparing MSG-N vs. Control-N, (* when p value <0.05, ** when p value <0.01, *** when p value <0.001).

Serum urea level was increased significantly in MSG-A and MSG-N groups as compared to control-A and control-N respectively. There was no significant difference in serum urea levels between MSG-A and MSG-N groups.

Table (4): Changes in serum levels of liver and kidney function tests (serum AST, serum ALT, serum urea and serum creatinine) among the study groups

	Control-A	MSG-A	Control-N	MSG-N
AST levels (U/L)	17.71 ±1.07	26.50±1.99 ^{a**}	18.49 ±1.86	29.71± 2.03 ^{b**}
ALT levels (U/L)	5.15 ± 0.31	7.71 ±0.82 ^{a*}	6.26 ±0.48	10.16 ± 0.49 ^{b***, c*}
Urea levels (mg/dl)	20.50± 1.14	34.85 ±1.73 ^{a***}	18.80 ± 1.41	30.14 ± 1.67 ^{b***}
Creatinine levels (mg/dl)	0.43 ± 0.04	0.64 ±0.04 ^{a**}	0.36 ±0.04	0.66 ± 0.03 ^{b***}

Data shown are mean ± SEM.^a Comparing MSG-A vs. Control-A,^b Comparing MSG-N vs. Control-N,^c Comparing MSG-A vs. MSG-N (* when p value <0.05, ** when p value <0.01, *** when p value <0.001).

Serum creatinine was increased significantly in MSG-A as compared to control-A and was significantly increased in MSG-N as compared to control-N. There was no significant change between MSG-A and MSG-N groups.

6.10. Histopathology results:

H&E and PAS liver sections of MSG-A group (fig.2) revealed dilatation of portal vein branches, vacuolar cytoplasmic degeneration of hepatocytes, lobular mononuclear inflammatory cell infiltrate and decrease in PAS staining of most hepatocytes.

H&E and PAS liver sections of MSG-N rats (fig.3) revealed portal vein branches dilatation in addition to foci of sinusoidal congestion and RBCs stasis, portal and lobular mononuclear inflammatory cell infiltrate. Scattered hepatocytes are shrunken and showed small pyknotic hyperchromatic nuclei with dense eosinophilic cytoplasm (Acidophilic bodies /apoptotic hepatocytes) indicating that liver affection is more when MSG is given in younger age group.

As regarding kidney sections of MSG-A group (fig.4), it showed patchy interstitial inflammatory infiltrate, cellular and hyaline tubular casts, vascular congestion and loss of PAS positive brush

borders in the proximal tubules. Sections from MSG-N group (fig.5) showed heavy interstitial inflammatory infiltrate, interstitial hemorrhage, cloudy degeneration of the tubular lining and focal necrosis indicating more damaging effect of MSG- in younger age. The changes in kidney tissue are patchy and mild affecting less than 25% of the kidney tissue

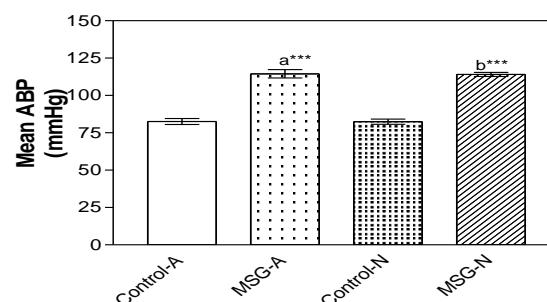


Fig. (1): The mean value of ABP along the study groups.

Data were expressed as mean ±SEM. a Comparing MSG-A vs. Control-A, b Comparing MSG-N vs. Control-N (* when p value <0.05, ** when p value <0.01, *** when p value <0.001).

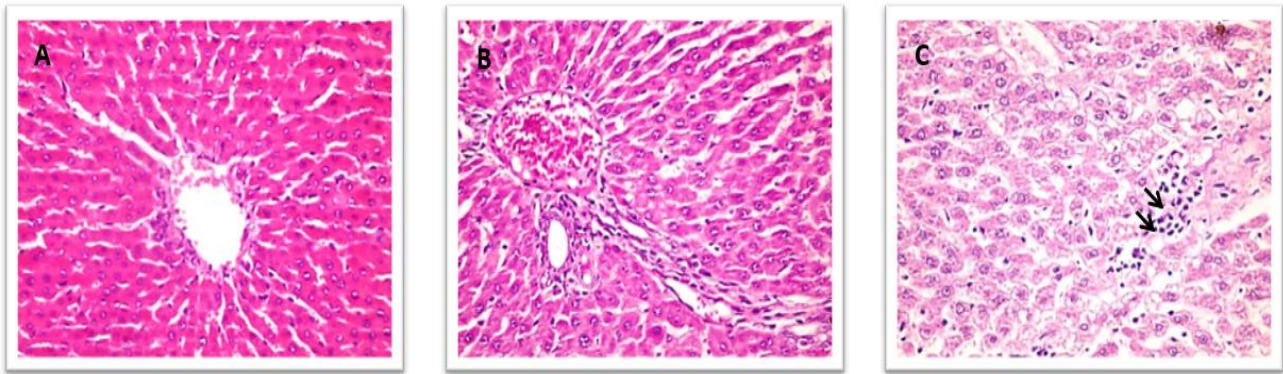


Figure (2): Histopathology of adult liver tissue; (A): Sections from liver tissue of adult rat control group, H&E stain (x400). (B,C): Liver tissue from adult MSG treated rats. (B): H&E stain (x400), Liver tissue showing dilatation of portal vein branches. (C): H&E stain (x400), Liver tissue showing Vacuolar cytoplasmic degeneration of hepatocytes and inflammatory cell infiltrate within the liver lobule (arrows).

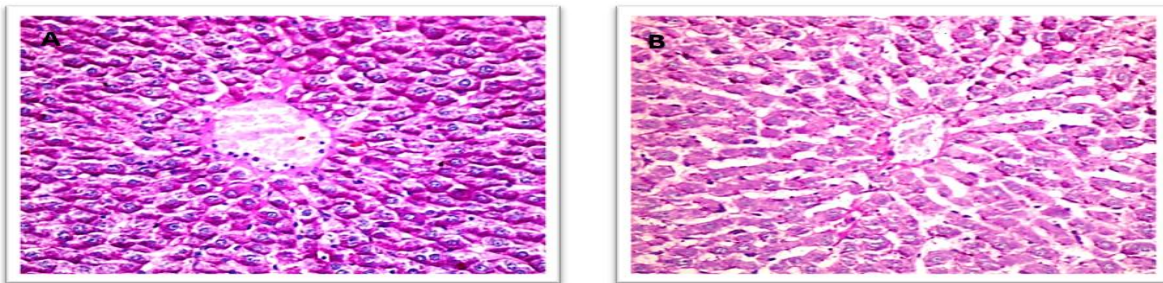


Figure (3): A: Sections from liver tissue of adult rat control group, PAS stain, x 400. B: Liver tissue from adult MSG treated rats showing decreased PAS staining in most hepatocytes, PAS stain, x400.

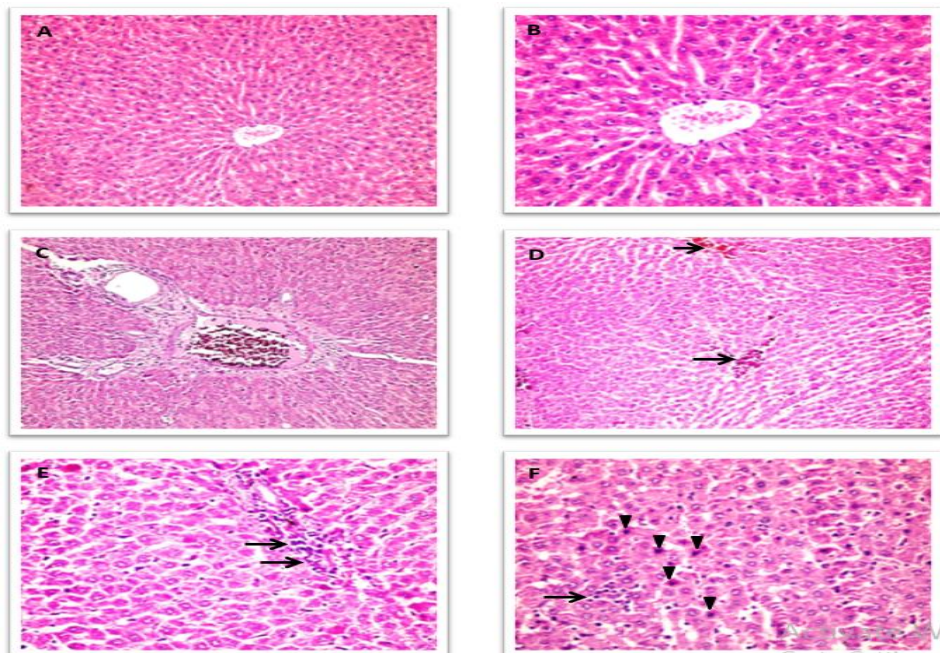


Figure 4: Histopathology of liver tissue of neonate rats. (A,B): Sections from neonate rats control group; A: H&E stain (x200), B: H&E stain (x400). (C-F): Sections from MSG treated neonate rats; C : H&E stain (x200) Portal vein branch dilation and congestion, D: H&E stain (x200) Focal sinusoidal congestion (arrows). E: H&E stain (x400) Portal inflammatory cell infiltrate (arrows) is seen. (F): H&E stain (x400) Lobular inflammatory cell infiltrate (arrows) and scattered hepatocytes with small hyperchromatic nuclei are present (arrow heads).

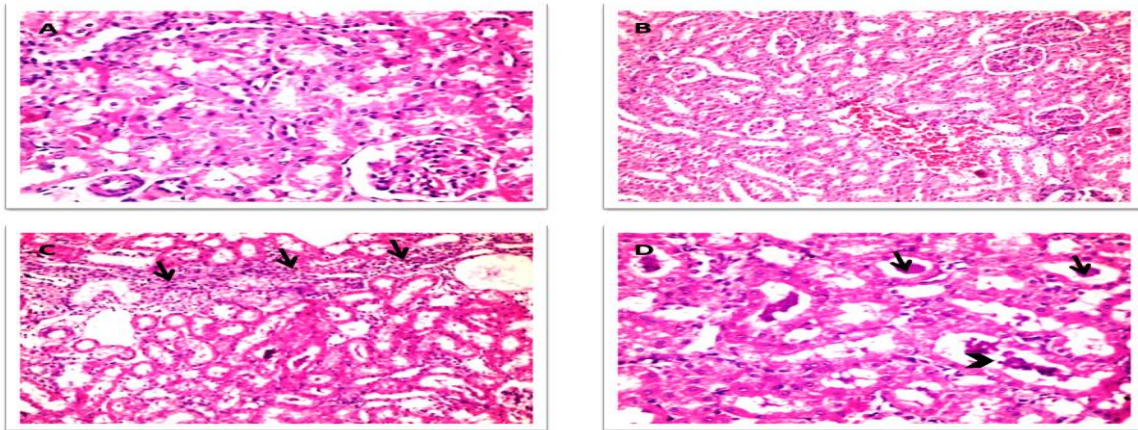


Figure (5): Histopathology of kidney tissue of adult rats; (A): Sections from adult rat control group; H&E stain (x400). (B-D) Sections from adult rat MSG group; (B) H&E stain (x200) showing vascular congestion. (C) H&E stain (x200) showing interstitial inflammatory infiltrate (arrows). (D) H&E stain (x400) showing hyaline casts (arrows) and cellular casts (arrowheads) in the renal tubules.

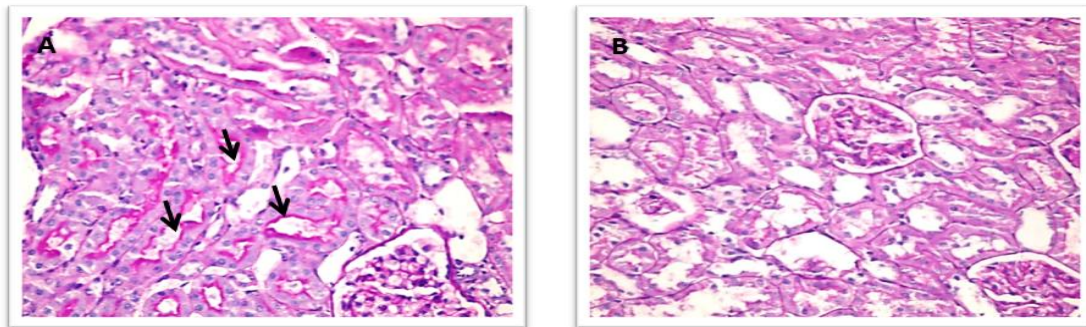


Figure (6): Histopathology of kidney tissue of adult rats; A: Sections from adult rat control group, PAS stain (x400) demonstrating positive brush borders (arrows) in the proximal tubules. B: Sections from adult rat MSG group. PAS stain (x400) demonstrating loss of brush border in proximal tubules.

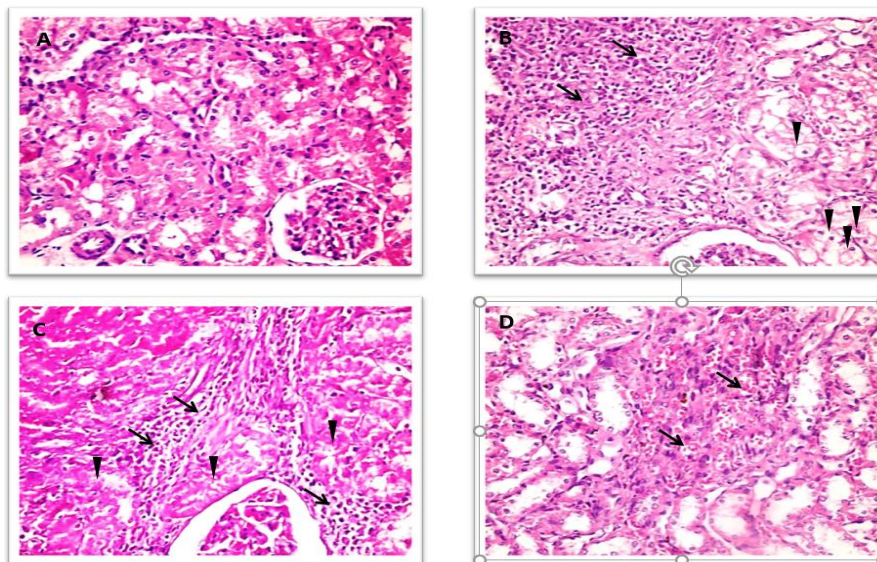


Figure (7): Histopathology of kidney tissue of neonate rats. (A): H&E (x400), Section from control group. (B-D) Sections from MSG treated neonate rats; B: H&E (x400), Heavy interstitial inflammatory cell infiltrate (arrows) and vacuolar cytoplasmic degeneration of the tubules (arrowheads). C: Interstitial inflammatory cell infiltrate (arrows) and focal necrosis of the tubular lining (arrowheads). D: Interstitial haemorrhage (arrows).

7. Discussion:

There is a major increase in the use of food additives currently; it can be harmful if they are consumed on the long run [17]. One of the major food additives widely used as flavor enhancer is MSG. There have been evidences that a complex relationship exists between MSG and the regulation of body weight and other metabolic parameters; a relationship that is modulated by age at time of exposure (neonates or adults) [18]. As regarding metabolic effects of MSG it was suggested that MSG caused disruption of the brain hypothalamus areas controlling body mass and energy metabolism was strongly involved in inducing several metabolic diseases in the MSG induced animal model. MSG has a neurotoxic effects and damage to cells in the hypothalamic arcuate nucleus (ARC) and closer areas. ARC neurons are crucial for the regulation of metabolic homeostasis, including insulin secretion and action and disruption of leptin signaling pathway and leptin resistance. It is suggested from our study that MSG can induce organ damage and toxicity in liver and kidney through its inflammatory effect (evidenced by high levels of TNF in serum) and its oxidant effect (evidenced by low levels of total antioxidant capacity in serum of rats administered MSG). It induced oxidative stress in organs.

The current study used I.P injection as the pharmacokinetics of substances administered intraperitoneally are more similar to those seen after oral administration, because the primary route of absorption is into the mesenteric vessels, which drain into the portal vein and pass through the liver. Therefore, substances administered intraperitoneally undergo hepatic metabolism before reaching the systemic circulation to study

the effect on liver and to ensure high levels of MSG in the liver and CNS.

Our results revealed increased the final BW, WG and BMI in MSG-A as compared to control-A. This was in agree with **Shukry et al.[17]** and **Khodier et al. [19]** who reported that MSG intake in adult rats for 3 months increased final BW. They stated that MSG intake caused impaired energy balance by increasing the palatability of diet and disturbing the leptin-mediated hypothalamic signaling pathway leading to obesity.

Results showed increased final BW, WG, WG %, BMI and Lee index in MSG-N as compared to control-N of similar age in accordance with **Fouda et al. [10]**. MSG treatment in rats resulted in endocrine abnormalities and obesity based on the greater percentage of fatty tissue in the intra-abdominal, femorogluteal regions, and Lee's index of obesity.

In addition, **Quines et al. [20]** showed that animals that received neonatal injections of MSG had Lee index higher than 0.3 at postnatal day 30 and this remained until 90 days of life. They explained that neonatal administration of MSG to male rats induced hypothalamic neurotoxicity confirmed by increased the levels of protein kinase C (PKC) phosphorylation in hypothalamus, which lead increased nuclear factor kappa-B (NfκB) levels, indicating a hypothalamic damage and dysfunction.

Results of fasting blood glucose levels revealed a significant increase in blood glucose level in MSG-A as compared to control-A and a significant increase in MSG-N as compared to control-N, while there was no significant difference between MSG-A and MSG-N. These

results were in agree with **Ceglarek et al. [21]** who showed that SC neonatal administration of MSG was associated with higher blood glucose level by 35% than control. **Quines et al. [20]**, explained increased blood glucose level by the decrease in the membrane glucose transporter 4 (GLUT4) content in the skeletal muscle, which probably reduces the glucose uptake. **Bako et al. [22]** showed that increased accumulation of fat in the adipose tissues in MSG induced obesity lead to inflammation. It was believed that these effects caused as result of MSG toxicity, which lead to stimulation of gluconeogenesis from glutamate and glutamine. It was suggested a possible deterioration of glucose tolerance in rats following MSG administration that could be attributed to decrease cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG [23].

Serum insulin levels and HOMA-IR levels in our study revealed hyperinsulinemia and insulin resistance in both MSG-A and MSG-N as compared to control-A and control-N groups respectively with significantly higher results in MSG-N as compared to MSG-A. These results were in accordance to **Khodier et al. [19]**, who reported that MSG changed levels of adipocytokines that could impair insulin action, increase breakdown of lipid and change the storage of triglycerides, resulting in increased flow of free fatty acids (FFA) and raised lipid stores in the liver and muscle, besides resistance to insulin hormone, leading to hyperglycemia and hyperinsulinemia.

In our results, serum leptin levels were significantly increased in both MSG-A and MSG-N as compared to control groups. This was in agreed with studies that linked MSG treatment in

experimental animals with hyperleptinemia and leptin resistance. The increase observed in leptin levels in our study was also in line with that reported by **Sáinz et al. [24]** who described that obesity linked to high levels of circulating leptin. Our results were also in agree with **Tomankova et al. [25]** that showed increased serum leptin levels after MSG neonatal treatment of mice.

As regarding Mean ABP, It was significantly increased in MSG-A as compared to control-A and in MSG-N as compared to control-N. There was no significant difference in mean ABP between MSG-A and MSG-N groups respectively. Our results were in agree with **Fouda et al. [10]** who observed a significant increase of renal and heart compression by the fat around and renal mass loss. This compression of the main renal arteries, veins and parenchyma resulted in an acute decrease of renal perfusion pressure, which was accompanied by decrease in renal blood flow, glomerular filtration, and fall in tubular fluid dynamics and urinary excretion. This induced a significant increase in the renal secretion of renin and plasma renin activity.

As regarding serum levels of TNF- α , they were significantly higher in MSG-A rats as compared to control-A. In addition, it was significantly higher in MSG-N as compared to control-N rats. There was no significant difference in serum levels of TNF- α between MSG-A and MSG-N.

These results were in agreed with **Khodier et al. [26]** that showed a marked increase in serum TNF- α levels compared to control in MSG treated adult rats. They concluded that the accumulation of pro-inflammatory cytokines and chemokines within the adipose tissue played an important role

in insulin resistance through obstructing inflammatory signaling and infiltration of immune cells in the adipose tissue.

Serum levels of TAC in our study, there was a reduction of TAC in both MSG-A and MSG-N groups as compared to control-A and control-N respectively. This was in agree with **Moen et al. [23]** they showed that MSG treated adult rats showed a significant reduction in some antioxidant markers (Catalase, TAC, total glutathione content and reduced glutathione). **Kodeir et al.[19]** showed a significant increase in lipid peroxidation upon MSG treatment in their study. This may suggest membrane disruption and alteration in cellular functions which lead to extreme decline in (GSH, SOD, and CAT) caused by MSG which may altering the antioxidant defense system and causes oxidative stress.

As regarding liver weight parameters in our study the final liver weight was found to be non significant in MSG-A as compared to control, while the final liver weight was found to be significantly increased in MSG-N as compared to control-N. There was no significant difference in liver index among the studied groups.

In agree with our results, **Konopelniuk et al. [27]** showed no significant change in relative organ weights (liver index) in MSG treated neonates.

As regarding liver enzymes AST and ALT, our results showed significantly elevated levels of both enzymes in both treated groups MSG-A and MSG-N as compared to control-A and control-N groups with no significant difference between MSG-A and MSG-N in AST level while there is mild significant elevation in serum level of ALT in MSG-N as compared to MSG-A.

Our results also showed that young age groups could be more vulnerable than adults as regarding exposure to MSG. Our results were in agree with **Shukry et al. [17]**, which showed that ingestion of MSG to adult male rats could significantly increase the levels of liver enzymes ALT, AST due to the cytotoxic effect of MSG that resulted in damage to liver cells and canaliculae leading to release of these enzymes in the circulation.

Our results concerning kidney weights showed a significant increase in final kidney weights in both MSG-A and MSG-N rats with no significant difference between both MSG treated groups. There was no significant difference in the kidney index (relative organ weight) in all study groups.

These results were in agree with **Contini et al. [8]** that showed a significant increase in final kidney weight in MSG treated adult Wistar rats. This was due to renal sodium handling in MSG rat and altered histoarchitecture, increase tubule-interstitial fibrosis, glomerular hypercellularity, tubular swelling and infiltration of inflammatory cells in rat kidneys.

As regarding renal function tests urea and creatinine, our results showed significantly elevated levels of both enzymes in both treated groups MSG-A and MSG-N as compared to control-A and control-N groups with no significant difference between MSG-A and MSG-N in urea or creatinine levels. These results were in agree with several studies that showed that MSG can affect both age groups whether given at neonatal or adult age proving to have a nephrotoxic effects.

Sharma.[28] explained the nephrotoxic effects of MSG by several mechanisms including the formation of ROS in the kidney exposed to MSG

was considered a major contributor to the nephrotoxic effects leading to cellular and functional damage.

As regarding histopathology of liver, our data showed that administration of 120 mg/kg/day MSG I.P for three months in MSG-A group revealed dilatation of portal vein branches, vacuolar cytoplasmic degeneration of hepatocytes, lobular mononuclear inflammatory cell infiltrate and decrease in PAS staining of most hepatocytes. On the other hand, the same dose for similar duration in MSG-N group caused portal vein branches dilatation in addition to foci of sinusoidal congestion and RBCs stasis, portal and lobular mononuclear inflammatory cell infiltrate. Scattered hepatocytes are shrunken and showed small pyknotic hyperchromatic nuclei with dense eosinophilic cytoplasm (Acidophilic bodies /apoptotic hepatocytes). This may indicate that MSG has a more serious effect when given during neonatal period.

These results were in agreement with previous studies that reported that administration MSG caused variable degrees of hepatocyte degeneration in the form of swollen hepatocytes and vacuolated cytoplasm as well as signs of cellular necrosis in the form of dark shrunken pyknotic nuclei (**Hejazi et al. [29], Elbassuoni et al. [30] and Shukry et al., [18]**). Mononuclear inflammatory cell infiltrate was also noted (**Elbassuoni et al. [30] and Shukry et al. [17]**). Dilated congested portal veins (**Hejazi et al. [29] and Elbassuoni et al.[30]**) as well as dilated central veins (**Onalapo et al. [31]**) were also reported. **Dosuky et al. [32]** noted markedly dilated central vein and the blood sinusoids.

As regards kidney histopathology, we found that kidney tissue from MSG treated adult rats showed patchy interstitial inflammatory infiltrate, cellular and hyaline tubular casts, vascular congestion and loss of PAS positive brush borders in the proximal tubules. Kidney tissue from MSG neonatal rats showed heavy interstitial inflammatory infiltrate, interstitial hemorrhage, cloudy degeneration of the tubular lining and focal necrosis.

The results agreed with other studies that reported that MSG administration led to variable degrees of degeneration and necrosis of renal tubules with hyaline and cellular casts (Osman et al. [33], Sharma et al. [28], Elbassuoni et al.[30], Othman and Bin-Jumah.[34], Kodier et al.[19]). Vascular congestion was also observed (Othman and Bin-Jumah.[34] and Kodier et al. [19]). Sharma et al.[35] documented the presence of interstitial inflammatory infiltrate and interstitial fibrosis in MSG treated rats.

In conclusion, the present study revealed that MSG has a definite metabolic influence as regarding obesity, hyperglycemia, and hyperinsulinemia with a definite insulin resistance. The results of the study also revealed the role of MSG in inducing inflammatory response and oxidant effect through decrease in TAC in serum. Our study also showed hepatotoxic and nephrotoxic effect of MSG indicated by chemical and histological analysis. The study also concluded that MSG could affect the younger age groups more than the older ages, so the cautious consumption of MSG in diet should be considered in infant, children and young age groups.

Recommendations

Based on our study, we recommend limitation of the MSG intake in diet in general and specifically limit the use to minimal doses in patients or people at risk of metabolic diseases, diabetes, hypertensive patients or patients with hepatic or renal affection. In addition, we recommend limitation of usage for young age groups (infants and children). The assessment of MSG impact on fetal neurodevelopment, following chronic exposure to dietary doses, represents valid research directions that should be considered.

Limitation of the study

Further time needed to study the long-term effect and chronic use of the MSG on the organ toxicity. In addition, the use of different methods of administration to assess whether these findings are relevant to human disease. In addition, Molecular mechanisms of action are needed to be investigated to understand the genetic bases of the metabolic and toxic effect of the MSG on health.

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