International Journal of Agriculture and Biosciences 2024 13(2): 76-84.
https://doi.org/10.47278/journal.ijab/2024.096

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Impact of Monosodium Glutamate on the Liver of Chick Embryo: Histology, Biochemical Properties and *AVBD9* Gene Expression

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ABSTRACT

Monosodium glutamate (MSG) is commonly used as a flavoring agent (E621) to increase food flavor, but it is still considered a controversial food additive. The present study aimed to evaluate the impacts of various MSG doses and microscopic changes in the growing embryo's liver and *AvBD9* gene expression. Ninety fertile chicken eggs were divided into three groups. Each group contained 30 eggs. Before incubation, the eggs were injected into the air cavity with 0.1mL of the MSG solution (0.75mg and 1.0mg MSG/g egg weight) and the control group with distilled water. Hematoxylin and Eosin (H & E) staining was done for histopathology. The biochemical characteristics of amniotic fluid were examined using the photometry approach. Real-time PCR (qPCR) was used to express the immunity gene *AvBD9.*Significant reductions in whole body weight, length, and deformities were observed in several MSG-treated chick embryos. By day 14, these embryos exhibited reduced hepatocyte density, small necrotic areas, lipid droplets, lymphocytic infiltration, minor endothelial lining ruptures, and dilated sinusoids, indicating slight disruptions in liver architecture. Quantitatively, the number of normal hepatocytes was significantly lower in treatment groups A (39.4±1.14) and B (36±1.58) compared to the control group C (48.6±1.52) ($P<0.001$). Furthermore, levels of liver enzymes such as alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were markedly increased in the treated groups. Additionally, there was a decrease in the expression of the *AvBD9* gene, underscoring the adverse impacts of MSG on growth, liver pathology, and *AvBD9* gene expression in chick embryos.

Keywords: Monosodium glutamate; Chick embryo; Liver; Histology; Gene expression

Article History
Article # 24-600
Received: 29-Apr-24
Revised: 27-May-24
Accepted: 30-May-24
Online First: 03-Jun-24

INTRODUCTION

The chemical name for monosodium glutamate (MSG) is AJI-NO-MOTO. It is frequently used as a food additive (E621) to enhance the flavor of many food products, including canned vegetables, soups, and processed meats (Gottardo et al., 2022; Moldovan et al., 2023). Nevertheless, it is regarded as a controversial component of food (Singh et al., 2015). MSG, like other amino acids, undergo Maillard-type reactions in the presence of reducing sugars, enhances flavor, and facilitates the production of food colors. The acceptable daily intake (ADI) rate for MSG is 30mg/kg body weight per day, as determined by the quantity of MSG that has been proven to have no harmful effects (Mortensen et al., 2017). However, most food manufacturers have utilized more MSG than necessary to make their goods more popular in recent years, especially in baby foods (Hossain et al., 2020). Overconsumption of MSG has been linked to pathological disorders such as addiction, stroke, epilepsy and retinal damage and

Cite this Article as: Islam MS, Ferdous KA, Amin MN, Kamruzzaman M and Islam MA, 2024. Impact of monosodium glutamate on the liver of chick embryo: histology, biochemical properties, and AVBD9 gene expression. International Journal of Agriculture and Biosciences 13(2): 76-84. https://doi.org/10.47278/journal.ijab/2024.096

A Publication of Unique Scientific Publishers

neurotoxic effects that negatively impact the liver and its functions (Eweka et al., 2010). Recent preclinical and clinical research increasingly suggests that monosodium glutamate may adversely affect human health, contributing to issues such as metabolic syndrome, neurotoxicity, renal toxicity, cardiovascular diseases, infertility, fetal underdevelopment, cancer, and immune system dysfunction (Yang et al., 2023; Mondal et al., 2024). According to particular research, MSG harms the liver in adult or newborn mice (Farr et al., 2010). Bhattacharya et al. (2011) demonstrated that giving neonatal mice 2mg/g of MSG damages the liver tissue. Metabolism and elimination of MSG from the body depends significantly on the liver. It is a vital organ; the liver carries over 500 fundamental biological activities. These include purifying the bloodstream of wastes and foreign objects, controlling blood sugar levels, and producing essential nutrients. Several enzyme functional assays, including alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST), have been developed to investigate the state of the liver.

In a study by Al Hargan et al. (2021), monosodium glutamate (MSG) was found to alter the expression of the *APC, BECN1*, and *TP53* genes in SW620 and SW480 colon cancer cell lines.*AvBD9* is a key component of the poultry immune system and has a consistent molecular structure and various functions. Xiao et al. (2004) and Lynn et al. (2004) employed a bioinformatic methodology to discover chicken *AvBD9*. *AvBD9* is widely distributed in several avian tissues, such as the liver, gallbladder, kidney, and genitourinary. The expression of the immunogenic component *AvBD9* may be detected utilizing the tissue of a chick embryo liver. Non-communicable diseases (NCDs) account for 70% of worldwide deaths and are the primary causes of poor health on a global level. Non-communicable diseases (NCDs) pose a substantial obstacle to reducing disparities in health at both the global and national levels (Di Cesare et al., 2013; Nugent et al., 2018). NCD disease death prevalence increases day by day due to individuals choosing to eat at restaurants because of their busy schedules and as parents also feed their children ready-to-cook foods from restaurants.

Although some investigations have shown that MSG has adverse effects on the liver, is linked to obesity in mice, and can pass on hepatic microstenosis to offspring, people are not aware of the consumption of MSG (Zanfirescu et al., 2019; Chaouche et al., 2024). While several studies have examined the effects of MSG, more research needs to be done specifically focused on its impact on embryos. Those researchers did not investigate the impact of MSG ingestion on the development of embryos. The chick embryo has been suggested as a growing animal model in embryology and developmental biology (Ribatti & Annese, 2023). The present study aimed to compare microscopic changes in the liver and AvBD9 gene expression of developing embryos and compare the effects of different doses of MSG. It might be possible to determine its precise impact on human embryo growth if pregnant women take it. Such data might be used to build a baseline of knowledge for nutritionists and public health professionals to reduce the harmful effects of MSG.

MATERIALS & METHODS

Ethical Approval

The written consent was taken from the Ethical committee of Institute of Research and Training, Hajee Mohammad Danesh and Technology (HSTU/IRT/3923) for the strategy to maintain the chick embryo in laboratory research and sample collection.

Chemicals

Commercial chemical supplier ZH Chemical, Dhaka, supplied Monosodium Glutamate (MSG)® (Cat # 010297, Korea) in powder form.

Hatching Eggs

Fertile chicken eggs (Sonali cross-breed) with an average egg weight of 53gm were purchased from the Nijam Poultry Farm, Bochagonj, Dinajpur-5200.

Experimental Design

There were 90 eggs in total, separated into three groups. Each batch consisted of 30 eggs.

Group A: Before incubation, 0.1mL of MSG solution (0.75mg MSG/gm egg weight) was injected into the air cavity of the viable egg in treatment group A. The weight of the embryos and gross and histological alterations were observed.

Group B: Before incubation, Group B was injected into the air cavity with 0.1mL of MSG (1.0mg MSG/gm egg weight). Embryonic weight, gross, and histopathological changes were also observed.

Group C: 0.1mL of distilled water was injected into the air cavity of control group C. Embryonic weight, gross, and histological changes were observed.

The incubator automatically controlled temperature, humidity, and forced air ventilation. Furthermore, it had distinctive egg holders that could turn the eggs automatically at a set rate of twelve per day. The eggs were rotated routinely every two hours, and the growth of the embryos was regularly monitored with an egg Candler. At 37.5°C, the temperature and humidity were set at 60%.

Collection of Specimens and Measuring Body Parameters

The embryos were euthanized using a chilling method on days 7, 10, and 14 after the recovered eggs were cleansed with a saline solution (Jacobsen et al., 2012). The eggs from the experiment were dissected at the larger end, and the embryos were carefully analyzed. Samples were collected on days 7, 10, and 14 to measure body weight and total length. A sample of the amniotic fluid was collected on the 14th day after MSG treatment. The liquid portion obtained after the amniotic fluid was separated by centrifugation was gathered for biochemical examination. The levels of the enzymes ALP, AST, and ALT were analyzed. The total body weights and length of the body were calculated using a digital weight measuring scale with a range of 0.1mg to 220g and a measuring scale in centimeters. Liver samples that were precisely selected were obtained. The liver samples of chicken embryos were fixed using 10% formalin for histopathology.

Histopathological Studies of Liver

The tissues were extracted from formalin and dehydrated using a series of ascending alcohol concentrations (50, 70, 90, 95, 100, and 100%) (Suvarna et al., 2019). One hour was allocated for each level of alcohol concentration. After dehydration, the tissues were immersed in xylene-1 and xylene-2 for ninety minutes each (Islam et al., 2019). After that, the tissues were immersed in liquid paraffin for two hours at 60°C. Paraffin blocks were created once the tissues were preserved entirely in paraffin. The paraffin blocks were sectioned using a microtome with a thickness of 5 µm. (LEICA RM2125 RTS, USA). Following dividing the paraffin block into slices, the slices were elongated by immersing them in warm water in a water bath heated to a temperature of 50°C. Subsequently, the tissues were positioned onto a pristine glass slide.

The glass slides were left to cure the next day. Once the slides had dried, Hematoxylin and Eosin (H & E) staining was performed. Afterward, the sections were coated with a cover slip using adhesive Canada balsam (Drury et al., 1983). Richter A Ptica biological microscope, specifically the U-2T model from Carlsbad, California, was utilized with an Amscope (MA500) to obtain high-resolution photos of the tissues in the targeted regions. The images were taken using 40× and 100× microscopic lenses. The quantification of hepatocytes in liver sections was performed using was performed using Image J software (Curvo et al., 2020). Following Islam et al. (2023), a predetermined field length was employed for cell counting. The cell counts in a field of a given length was used to indicate the total number of cells. Measurements and cell counts were conducted on five slides from each group. Hepatocytes that are considered normal are often characterized by their consistent size and well-defined cellular features.

Analysis of Amniotic Fluid for Biochemical Parameters

A syringe with an 18-gauge needle was used to collect amniotic fluid on the 14th day of the incubation process. A total of five samples were collected from each of the groups. The eggshell and shell membranes were initially removed at the end, holding the air sac. The allantoic membrane was removed to collect amniotic fluid. The liquid portion of the amniotic fluid was collected to assess chemical properties following a process of spinning with a force of 3000g and acceleration due to gravity for 15min. The levels of ALP, AST, and ALTwere quantified using photometry on a Siemens Advia 1800 instrument from Germany (Siemens ADVIA Chemistry Reagent Kit, Cat # 09700055, 09700047, and 09700046).

Evaluation of AvBD9 Gene Expression for the Administration of MSG

The housekeeping gene *GAPDH* was used to see the gene *AvBD9* expression level. The used primers and gene sequences are as follows- *AvBD9*: forward primer- AACACCGTCAGGCATCTTCACA, reverse primer- CGTCTTCTTGGCTGTAAGCTGGA, product size- 131bp (Laptev et al., 2019); housekeeping gene *GAPDH*: forward primer- CCTCTCTGGCAAAGTCCAAG, reverse primer- GGTCACGCTCCTGGAAGATA, product size- 176bp (Khosravi et al., 2018). The expression level of the immunity gene AvBD9 in the treatment compared to the controls was assessed by quantitative real-time PCR (qPCR). In summary, the Monarch® Total RNA Miniprep Kit was used to harvest RNA from the liver of the chick embryo at day 14. (Cat # T2010S, New England, Biolabs Inc. USA). Following the manufacturer's instructions, the RNA was extracted (n=3 in each group), with a bit of alteration made by applying liquid nitrogen to the sample to crush and homogenize it.

In the Central Laboratory of the Hajee Mohammad Danesh Science and Technology University, Bangladesh, RNA purity (A260nm: A280nm) and concentration (ng/l) were assessed using NANODROP ONE (Thermo Scientific, USA). According to the manufacturer's instructions, cDNA was generated from extracted RNA using the ProtoScript® II First Strand cDNA Synthesis Kit (Cat No: E6560S, New England, Biolabs Inc. USA).

The qPCR reaction was conducted in the Molecular Biology Laboratory, Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Bangladesh, using EcoTM 48 Real-Time PCR (PCR max, Staffordshire, ST15 0SA, United Kingdom). Using a Sybr Green test and Luna Universal qPCR Master Mix, it was accomplished (Cat No: M3003S, New England, Biolabs Inc. USA). The thermal profile for qPCR is 95°C for 1min of holding, 95°C for 10s of denaturation (40 cycles), 60°C for 15s of annealing, and 72°C for 20s of extension.

Statistical Analysis

The raw data from the different parameters in the current study were encoded, inputted, and sorted using MS Excel before being subjected to statistical analysis. The One-Way Analysis of Variance (ANOVA) test was used to examine the data statistically. The Tukey's HSD (honestly significant difference) approach was used to compare two pairs of ANOVA results. It was significant at 0.001. The data were sorted and checked for duplication and missing values using cross-checking.

RESULTS

Congenital Disorders

Subcutaneous bleeding was also seen in various treated chick embryos on days 7 and 10 days (Fig. 1). The abdominal hernia was seen in the treated embryos on day 14 (Fig. 2). Delay in growth occurred in all the treated group of embryos as compared to the controls. Brain and beak deformities were seen in several treated embryos on day 10 (Fig. 2). The number of abdominal hernia and subcutaneous bleeding were seen more in treated group B than in treated group A.

Growth Parameters

Treated embryos substantially reduced both whole-body weight and whole-body length at days 7, 10, and 14 compared to the control group ($P<0.00001$). All the data were significant in Tukey's HSD (honestly significant difference) procedure when compared among the pairs of the ANOVA table. MSG caused retardation of growth significantly by significantly decreasing whole body weight

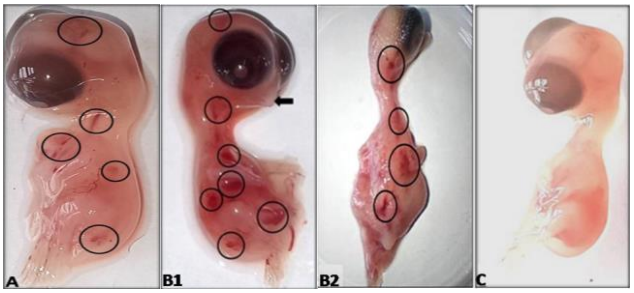

Fig. 1: Different congenital abnormalities in the treated group caused by MSG. A) treated chick embryos at the dose of 0.75mg/gm egg at day 7 with hemorrhage (circle); B1) treated chick embryos at the dose of 1mg/gm egg at day 7 with profuse hemorrhage (circle), beak deformities (arrow); B2) treated chick embryos at the dose of 1mg/gm egg at day 10 with profuse hemorrhage (circle) and C) chick embryos without administration of MSG showed no hemorrhage.

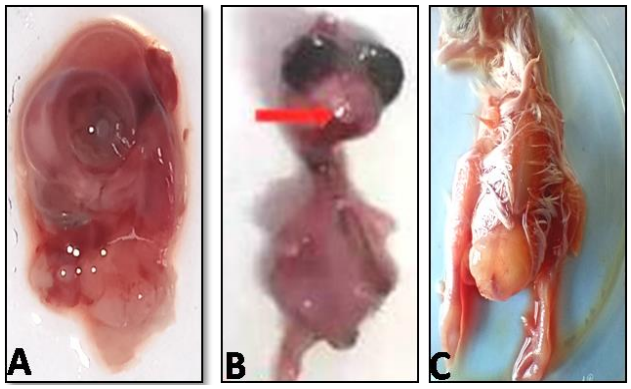

Fig. 2: Different congenital abnormalities in the treated group caused by MSG. A) treated chick embryos at the dose of 0.75mg/gm egg on day 7 with growth retardation; B) treated chick embryos at the dose of 1mg/gm egg on day 10 with malformation of the brain; and C) chick embryos with abdominal hernia at day 14 of incubation.

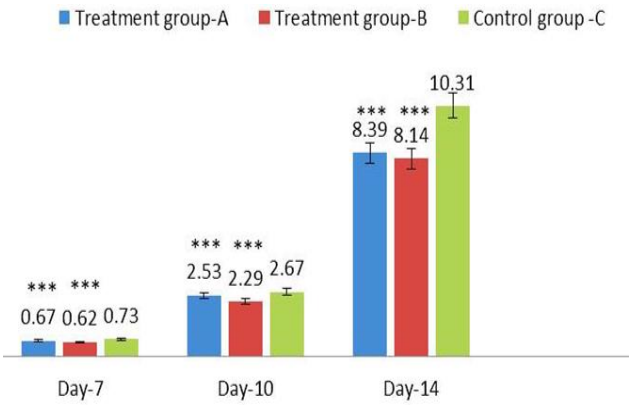

Fig. 3: Bar diagrams illustrating the impact of MSG on morphometric assessments of chick embryos- whole body weight. Compared to the control group, the treated group's total body weight decreased significantly. The body weight of group B was significantly reduced than group A. For each day group, n=7 samples were used to calculate means and standard errors. Statistical analysis indicates a notable distinction (***) between the treatment groups and the control group, as well as within the treatment groups themselves (P<0.001), confirming the influence of MSG on embryonic development.

and body length of chick embryos in groups A and B at days 7, 10, and 14 compared to controls (Fig. 3, 4). All the body growth parameters decreased significantly in treated group B compared to group A.

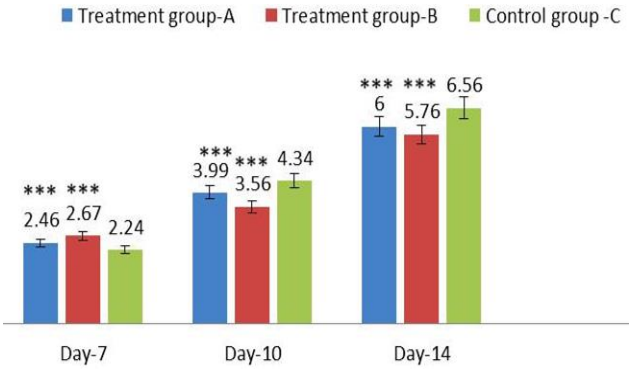

Fig. 4: Bar diagrams illustrating the impact of MSG on morphometric assessments of chick embryos- whole body length. The treated group's total body length decreased significantly compared to the control group. The body length of Group B was significantly reduced from that of Group A. For each day group, n=7 samples were used to calculate means and standard errors. Statistical analysis indicates a notable distinction (***) between the treatment groups and the control group, as well as within the treatment groups themselves (P<0.001), confirming the influence of MSG on embryonic development.

Biochemical Parameters of the Amniotic Fluid

The administration of MSG led to changes in the biochemical parameters of the amniotic fluid in chick embryos, as seen in Table 1. The concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were significantly elevated in comparison to the control group. The statistical analysis showed a significant difference (P<0.01). The embryos that received different dosages of MSG exhibited considerable variations among them.

Table 1: The amniotic fluid's biochemical characteristics in chicken embryos due to the administration of monosodium glutamate (MSG) on 14th day

Parameters	Control	Group A	Group B
ALP (IU/L)	6.08±0.86	252.98±6.85a	275.39±7.62b
AST (IU/L)	8.17±0.62	154.64±7.23a	178.16±6.94b
ALT (IU/L)	2.98± 0.58	52.85±4.26a	53.50±1.67b

In group n=5. Values (mean±SD) bearing different alphabets in a row differ significantly (P<0.001). MSG=Monosodium glutamate. Group-A=0.75mg MSG/gm egg weight; Group-B=1mg MSG/gm egg weight.

Effects on Liver Development

The liver in the control group of chick embryos consisted of the right and left lobes, with the right lobe being larger than the left. On day 14 of chick embryo development, the liver cross-sections of the treated groups revealed reduced hepatic cell density and more significant bleeding due to dilated venous canals than the untreated groups. The hepatic cords and connective tissue capsules in the control group were much more visible than in the treated group.

Tiny necrotic patches, light vacuolation, and a little disturbed liver architecture were observed in the 0.75mg/gm MSG-treated group on day 14. The congested central vein was seen, which was enlarged with a ruptured endothelial (RE) lining. In the treated liver tissue sections, necrotic regions (N), central veins (CV) containing RBCs, infiltrated lymphocytic infiltration (LI), and minor ruptured endothelial lining (RE) with dilated sinusoid (Fig. 5A, 5B).

After receiving MSG (1mg/gm), the chick embryonic liver was histologically photographed on day 14 and

observed that extensively ruptured endothelial (RE) lining with a dilated sinusoid, areas of necrosis (N), central vein (CV) containing RBCs, and highly invaded lymphocytic infiltration (LI) (Fig. 5C, 5D).

The total amount of normal hepatocytes was counted from the liver histological section at day 14 of incubation in 100× magnification. Table 2 illustrates the numerical value of normal hepatocytes in the control and different groups. The number of normal hepatocytes in treatment group A (39.4±1.14) and B (36±1.58) was significantly lower ($P<0.001$) than in control group C (48.6±1.52) (Fig. 5E, 5F).

Several tiny, transparent vesicles were found in the cellular cytoplasm of the peripheral region of the liver's histological section. Comparing the treated group to the control, the quantity and size of lipid droplets increased. The pyknotic nucleus of hepatocytes was seen in the liver of the treated group (Fig. 6).

AvBD9 Gene Expression

A qPCR assay was performed on day 14 of the incubation period to assess the expression of the gene *AvBD9*. The MSG-treated groups at dosages of 0.75 and 1mg/kg egg weight demonstrated a reduction in the mRNA expression of AvBD9 compared to the control group (Fig. 7).

Table 2: The number of normal hepatocytes in exposure (A and B) and control group (C)

Group	Control	Group A	Group B
Normal hepatocytes	48.6±1.52[a]	39.4±1.14[a]	36±1.58b

In group n=5. Values (mean±SD) bearing different alphabets in a row differ significantly ($P<0.001$). Group-A=0.75mg MSG/gm egg weight; Group-B=1mg MSG/gm egg weight.

DISCUSSION

MSG is the most popular and widely used taste enhancer. Numerous research studies were carried out to evaluate the adverse effects of MSG on the fetuses of pregnant women. These populations are particularly susceptible to MSG because their tissues develop quickly, and their immune systems take longer to mature (Al-Ghamdi, 2017; Shosha et al., 2023). It is naturally present in various natural foods and is utilized by numerous tissues, including the muscles, liver, and other organs (Munro, 1979; Garattini, 2000; Bayram et al., 2023).

MSG caused retardation of growth in whole body weight and body length in the treated chicken embryos of the present study. These results are agreed with the results of other research (Oforofuo et al., 1997; Al-Qudsi & Al-Jahdali, 2012; Tala'a, 2019; Shosha et al., 2023). These studies found that MSG caused delay and retardation of the growth of the embryos. It may be because the MSG causes slow growth of embryos through bleeding. As a result, embryonic cells were not able to grow properly.

Research using laboratory animals has been conflicting concerning the connection between MSG consumption and body weight. Whereas some indicated a negative relationship (Kondoh and Torii 2008), other studies revealed a significant linkage between MSG consumption and obesity (Hirata et al., 1997; Zanfirescu et al., 2019; Chaouche et al., 2024). The varying doses employed in the various studies could cause the observed variations. Numerous congenital deformities, including abdominal hernias, subcutaneous hemorrhage, and other issues, were observed in the study.

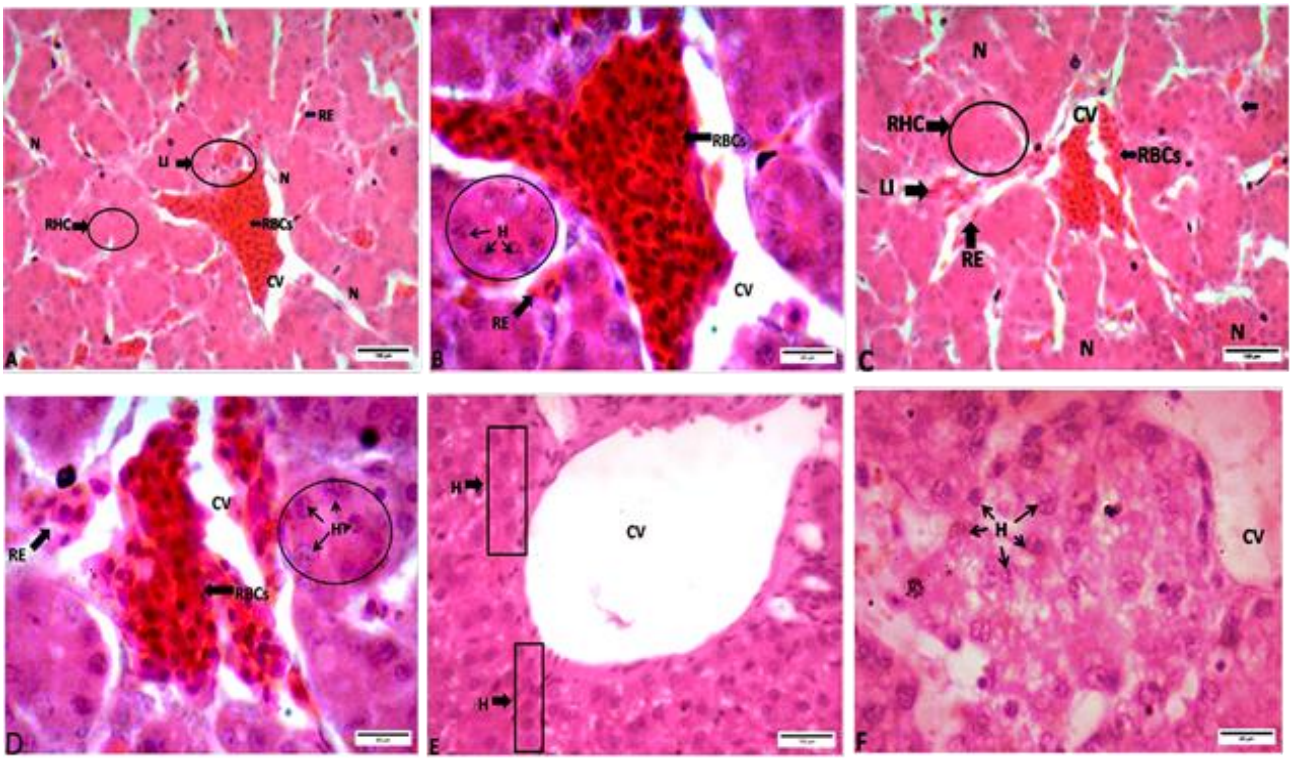

Fig. 5: Photomicrographs depicting liver abnormalities of chick embryos treated with monosodium glutamate (MSG) (A & B: 0.75mg/g and C & D: 1mg/g) on day 14. Treated groups exhibited ruptured hepatic cord (RHC) architecture, necrosis (N), central vein (CV) with RBCs, invaded lymphocytic infiltration (LI), and mild ruptured endothelial (RE) lining with dilated sinusoid. Fig. E and F were without MSG and showed central vein (CV) without RBCs and normal hepatocytes (H) with normal size and shape. H&E-stained sections. Scale bars: A, C & E=155µm, B, D & F=60µm.

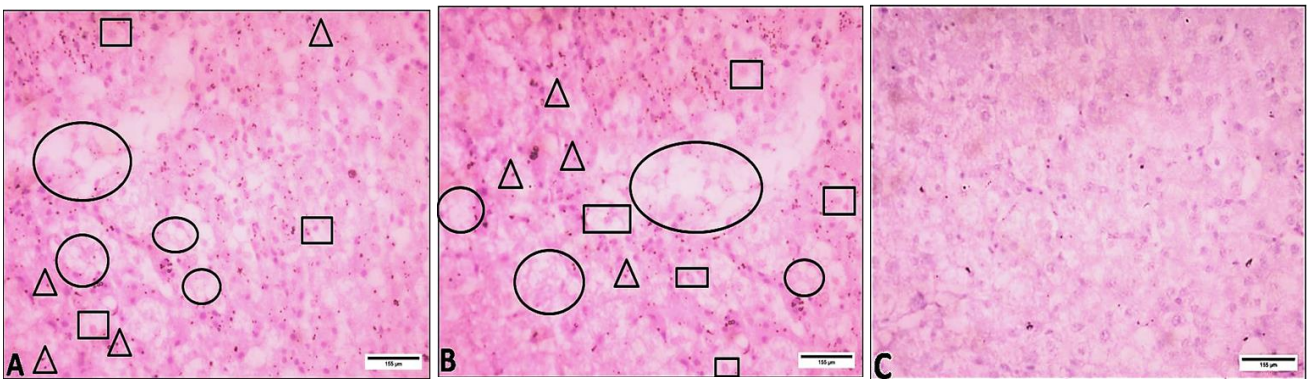

Fig. 6: A representative micrograph of peripheral part of liver (6, A) at day 14 of chick embryo liver after administration of MSG (0.75mg/gm) showed lipid droplet (in oval), cytoplasmic vacuolation (in square), pyknotic nucleus (in triangle) (H&E stained, 40×, scale bar = 155 µm); (6, B) at day 14 of chick embryo liver after administration of MSG (1mg/gm) showed more lipid droplet (in oval), cytoplasmic vacuolation (in square), pyknotic nucleus (in triangle) (H&E stained, 40×, scale bar = 155 µm); (6, C) at day 14 of chick embryo liver of control administration of distilled water showed pathological changes (H&E stained, 40×, scale bar = 155 µm).

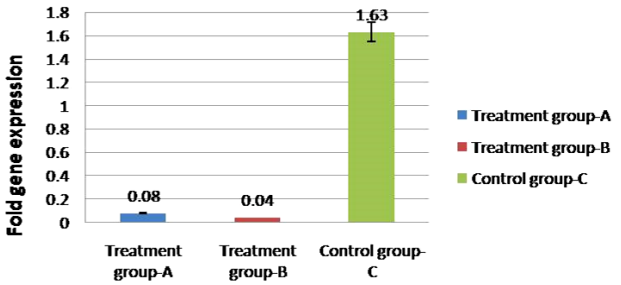

Fig. 7: Expression of AvBD9 gene due to administration of monosodium glutamate. The expression of the AvBD9 gene (n=3 per each group) reduced due to being treated with MSG as compared to the control.

Some other investigations revealed that MSG causes bleeding (Al-Qudsi & Al-Jahdali, 2012; Meraiyebu et al., 2012; Al-Ghamdi, 2017). Studies revealed that MSG also linked to monophathalmia (Wuu et al., 1988; Kawamura and Azuma, 1992; Sucher et al., 1997; Osborne et al., 1999; Vorwerk et al., 1999; Tamás et al., 2004). However, monophathalmia was not observed in the current investigation. It might be brought on by dosage variability. Congenital abnormalities were all brought on by insufficient blood supply and poor nutrition transfer from the yolk to the embryo.

The elevation in serum ALT, AST, and ALP levels in MSG-treated chick embryos, as noted by various researchers in rats, including Tawfik and Al-Badr (2012), Osman et al. (2012), Sharma et al. (2013), and Gad EL-Hak et al. (2021), indicates hepatocyte dysfunction or damage, potentially due to biliary pressure or disturbances in hepatic secretory activities, confirmed by histopathological examination.

The harmful effects of MSG on the liver have been reported in particular research. According to Bhattacharya et al. (2011) and Shosha et al. (2023) MSG has liver-damaging effects on newborn rats. The liver cross-section in the study's control group revealed identical alterations in prior investigations (Patten, 1971; El-Naggar, 1977; Abdel-fatah, 1992; Al-Gamdi, 2007; Al-Qudsi & Al-Jahdali, 2012). In this investigation, the liver sections revealed the same findings. Due to the dilated venous canals, the treated group experienced increased bleeding and less density of hepatic cells. It is comparable to the results of earlier investigations (Al-Qudsi and Al-Jahdali, 2012). According to the present study, injecting eggs with MSG solution (0.75mg and 1mg MSG/gm egg weight) resulted in less hepatic cell density in all study groups and age groups.

All treated groups showed hemorrhages in the central vein and sinusoids, as well as a few empty spaces in the peripheral liver regions. Other studies also found these modifications (Ortiz et al., 2006; Nakanishi et al., 2008; Al-Qudsi & Al-Jahdali, 2012). The study by Nakanishi et al. (2008), which used a more significant dose of 2mg/g of body weight administered five times, resulted in more severe findings. The treated group had numerous lipid droplets, the embryos in the treated group had pyknotic nuclei, and in the present study also found phagocytic cells in various blood sinusoids. It may be due to theMSG exposure disrupting the liver's ability to process fats, accumulating triglycerides, and forming numerous lipid droplets. MSG induces cellular stress, leading to cell death characterized by pyknotic nuclei—where the nucleus shrinks and condenses. An increased presence of phagocytic cells in the liver's blood sinusoids indicates an immune response to remove dead or damaged cells caused by MSG (Shosha et al., 2023). Other studies also noted these effects similar to the present study (Abdel-fatah, 1992; Ortiz et al., 2006; Al-Gamdi 2007; Nakanishi et al., 2008).

The genes *AvBD9* were expressed substantially less in the exposed group than in the control group in the present study. One likely idea for how MSG decreases immune gene expression is that exposure to MSG concentrations has a dose-dependent effect on B cell survival.mgluR-7 receptors are likely responsible for glutamate-induced apoptosis in memory and naive B cell types (Jovic et al., 2009). Similar to this, memory and naive cells express different glutamate receptors. Immune cells undergo oxidative stress and death due to different glutamate receptor expression patterns (Jovic et al., 2009). The MSG might also affect the activity of proteins that bind to DNA and reduce *AvBD9* expression.

Conclusion

In conclusion, the current study has provided substantial evidence of the detrimental effects of

monosodium glutamate (MSG) on the development and function of the liver in chick embryos. Histological examinations demonstrated notable changes such as decreased hepatic cell density, hemorrhages, and the presence of lipid droplets and pyknotic nuclei. These findings correlate with an increased level of liver enzymes, suggesting hepatocyte dysfunction and damage likely due to the toxic effects of MSG. Additionally, biochemical analysis revealed significant alterations in liver function markers, further confirming the adverse impact of MSG on liver health. Moreover, the study revealed a downregulation of the *AvBD9* gene, indicating potential suppression of immune-related gene expression due to MSG exposure. This suppression could be linked to the glutamate-induced apoptosis in B cells mediated through specific glutamate receptors, further underscoring the wide-ranging effects of MSG on embryonic development and immune function. Overall, these findings align with previous studies highlighting MSG's potential to cause developmental delays, congenital abnormalities, and liver damage. The results advocate for a cautious approach to the use of MSG, particularly in vulnerable populations such as developing embryos, and underscore the need for further research to fully understand the biochemical pathways affected by MSG exposure. This study contributes to the growing body of evidence on the biological impacts of dietary additives and their implications for developmental biology and toxicology. Further advanced studies should be performed to know the mechanism of MSG that causes adverse effects on embryonic and human embryonic development.

Conflict of Interests

The authors state that there are no conflicts of interest with this study.

Acknowledgment

The authors thank the Institute of Research and Training (IRT), Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, for financial support [grant number HSTU/IRT/3005/2020/91].

Authors Contribution

MSI, KAF, and MAI conceptualized and designed the experiment. MSI, MNA, and MK conducted the study. RH oversaw and coordinated the experiments and provided clinical data. MSI and MN analyzed the experimental data statistically. KAF and MAI drafted the manuscript. All authors contributed to the critical revision of the manuscript and approved the final version.

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