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Assessment of Monosodium Glutamate-induced histological and osteological injury in rats embryo and amelioration with pomegranate juice.

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ABSTRACT

Monosodium glutamate (MSG) is a flavor enhancer that appears as a small, white to off-white, odorless crystal powder. It can cause serious health problems as its metabolites can be toxic to many organs. the current study aimed to determine the toxicity of MSG on fetal *Rattus norvigecus domestica* and the ameliorating role of pomegranate juice (Pg. J) against it. *Punica granatum L.* (Pg) is a long-lived and drought-tolerant plant. It has been used in the traditional medicine of various civilisations as a “curative food”. The Pg tree offers several medicinal components, including its seeds, juice, peel, leaves, flowers, and root bark. Each part is linked to various health benefits, such as cancer prevention, reducing arteriosclerosis, and lowering high cholesterol levels.

Forty-two pregnant female were randomly divided into 6 groups (n=7): the control group "C" received orally distilled water; "G1" received orally/daily 10ml (Pg. J) /kg b.w(dissolved in distilled water; "G2" received orally/daily 0.55 g MSG /kg b.w; "G3" received orally/daily 0.55 g MSG /kg b.w with 10 ml (Pg. J) /kg b.w; "G4" received orally/daily 1.6 g MSG /kg b.w; and "G5" received orally/daily 1.6 gMSG / kg b.w with 10 ml (Pg. J) /kg b.w. All groups treated on the 1^{st} – 20^{th} days of pregnancy. The fetal length decreased significantly in groups "2,3,4,5". Major skeletal abnormalities in the fetuses included incomplete ossification of the skull, vertebrae, and pectoral and pelvic girdles with their fore and hind limbs in groups 2,3,4,5. MSG

has been shown to histopathologically alter the lungs and liver of fetuses. The high doses had the most chronic effect than the low doses.

keywords: Monosodium Glutamate, Pomegranate juice, Skeletal development ,Rat fetuses, Lungs, Liver.

1. INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of glutamic acid **[1,2,3].** Ingested glutamate can reach the body via dietary protein or meals high in free glutamate **[4].** MSG has been used to season food for over a century, and various studies have been conducted to ensure its safety. In addition, it is an important flavour enhancer in various fast meals **[5].** The body's main excitatory neurotransmitter is glutamate **[6].** MSG ($C_5H_8NNaO_4$) is chemically known as 2-amino pentane dioic or 2-amino glutamate **[3].** MSG is manufactured by glucose fermentation with a nitrogen source. It uses hydrolysis of molasses and starch **[4].**

MSG has been proven to be toxic for both humans and experimental animals **[6].** Several experimental investigations have revealed that MSG harms different organs, including the CNS, liver, kidneys, uterus, thyroid, spleen, thymus, and testes, and has a genotoxic impact **[7].**

The lungs are more vulnerable to oxidative damage than any other organ due to their continual exposure to air which may include harmful particles or oxidant gasses like ozone or nitrogen oxide **[8].** Treatment with a dose of 2 mg MSG /kg of b.w on the 19^{th} day of gestation had significantly slower lung development and growth retardation in fetuses compared to the control group. Additionally, discontinuities were observed in various epithelial sections **[8].**

Administration of MSG also has been shown to induce oxidative stress through induction of lipid peroxidation, reduction of glutathione (GSH), enhancement of the activities of superoxide dismutase (SOD), glutathione-s-transferase (GST), and catalase in the liver of the experimental animals which in turn, result in hepatotoxicity **[9].**

Punica granatum L. (Pg) is a long-lived and drought-tolerant plant **[10].** Pg has been used in the traditional medicine of various civilisations as a “curative food” for fever, ulcers, diarrhoea, acidosis, dysentery, haemorrhage, microbial infections, parasites and respiratory disorders **[11,12].** The useful effects of Pg are related to its extensive

spectrum of phytochemicals, such as tannins, alkaloids, and dyes **[13].** Pg fruit is rich in antioxidant ingredients which could be recommended as a protective constituent of a healthy diet against the adverse effects of stressful agents as MSG **[14,15].**

The current study aimed to determine the influence of MSG on fetal body length, skeletal structure, and histopathological alterations in the lungs and liver on the 20th day of pregnancy.

2. MATERIAL AND METHODS

2.1. Pomegranate Processing

Fresh pomegranates were washed, crushed, and squeezed. The juice was filtered, pasteurized, and stored at -18°C.

2.2. Chemicals

MSG (Monosodium Glutamate) has a molecular weight of 187.13 g/mol, a purity of 99%, and is identified by catalogue number S209112.

It was obtained from Egypt's Sigma Pharmaceuticals Manufacturing. The median lethal dose (LD_{50}) of MSG in rats is 15–18 g/kg b.w **[5].** The amounts of MSG dissolved in distilled water utilized in this investigation were 1.6 and 0.55 g MSG /kg b.w, which equals 1/10 and 1/30 of LD_{50}, respectively. Sigma Pharmaceutical Industries provided the remaining stains and lab supplies, including alizarin red S, alcian blue, Harris hematoxylin and eosin (H&E), glycerin, 95% ethyl alcohol, and 10% neutral formalin solution.

2.3. Animals

Theodor Bilharz Research Institute (Giza, Egypt) provided pure-strain virgin female and male albino rats (*Rattus norvegicus domestica*, weight: 200 ±20 g, ages: (8–12 weeks). They were housed in hygienic, air-conditioned cages with a 12-hour light/dark cycle, regulated temperature (25°C), and limitless food and drink. After a week to get used to the lab environment, male and female rats were mated in a 1:3 ratio. The presence of a vaginal plug was used to indicate day "1" of gestation. Guidelines for using animals in experiments were established by the Benha University's local ethics committee (ZD/FSc/BU-IACUC/2023-19) with permission numbers (BUFS-REC-2024-260 Zoo).

2.4. Experimental design

The pregnant rats were arranged into six groups, each group is consisted of 7 rats. the control group "C" received orally distilled water; "G1" received orally/daily 10ml Pg.J /kg b.w (dissolved in distilled

water); "G2" received orally/daily 0.55 g MSG /kg b.w; "G3" received orally/daily 0.55 g MSG/kg b.w with 10 ml Pg.J/kg b.w; "G4" received orally/daily 1.6 g MSG /kg b.w; and "G5" received orally/daily 1.6 g MSG /kg b.w with 10ml Pg.J/kg b.w. All groups are treated on the 1^{st}–20^{th} days of pregnancy.

2.5. External morphological analysis

On the 20^{th} day of pregnancy, the pregnant rats' uteri were removed via a hysterotomy, an open abdominal incision, and a laparotomy, which resulted in the birth of fetuses through caesarean sections. The fetuses were measured for length, and their morphology was checked for any outward abnormalities like swellings or red patches. Any morphologically noticeable abnormalities were also photographed for a more in-depth assessment.

2.6. Insights on the endoskeleton

After four days of immersion in 95% ethyl alcohol, fetal residue was removed with the addition of acetone for one day. To identify cartilage and bone in fetuses, alizarin red S and alcian blue staining were both utilized. 20 ml of staining solution (consisting of 17 volumes of 70% ethanol, 1 ml of alizarin red S in 95% ethanol, and an equal volume of filtered alcian blue in 70% ethanol) was used to stain each fetus's skeleton. The combination also contained 1 ml of acetic acid. The stained fetus was cleaned with water, and then placed in progressively higher concentrations of glycerol and 1% aqueous KOH solution until it was preserved in 100% glycerin **[16].**

2.7. Histopathological examination

A dorsal midline incision was made to gain access to the lungs and liver tissues to swiftly remove them. Samples of tissue were preserved in a neutral formalin solution (10%). Sections (5 µm) were stained with H&E for routine light microscopy histopathological analysis **[17].**

2.8. Statistical analysis

The Kruskal-Wallis H test was employed in the statistical analysis **[18]** to identify any remarkable variations in the independent variable of fetal lengths among the different groups. This was followed by the post-hoc Dunn's test **[19].**

3. RESULTS

3.1. Effect of MSG on the external morphology of rats' fetuses

MSG caused a substantial reduction in fetal lengths in treatment groups compared to the control group (Figures 1).

The Kruskal-Wallis H test demonstrated a significant difference between the control and fetal body lengths. Body lengths were considerably shorter in MSG-treated groups compared to the control group (Figure 2). The Kruskal-Wallis H test indicated that there was a significant difference in the dependent variable between the different groups, $\chi 2$ (5) = 28.67, p < .001, with a mean rank score of 31.36 for control, 36.71 for G1; 14.5 for G2; 22.93 for G3; 7.79 for G4 and 15.71 for Group5. The Post-Hoc Dunn's test using a Bonferroni corrected alpha of P<0.0002 indicated that the mean ranks of the following pairs are significantly different: C-G4, G1-G2, G1-G4 and G1-G5. G2 and G4 were the most affected groups.

The morphology of MSG-administered fetuses indicated the formation of superficial hematomas, which are evidence of congenital anomalies in various sections of the body. Red spots appeared on the bodies of G4 and G2 fetuses (Figure 1).

3.2. Effect of MSG on the endoskeleton of rats' fetuses

Albino rats' skeletal structure is formed of two sections: the axial and appendicular skeletons. The former includes the skull, spinal column, ribcage, and sternum. The pectoral girdle, forelimbs, pelvic girdle, and limbs comprise the latter (Figures 3, 4 &5). The administration of two dosages of MSG to the mother resulted in a variety of unfavorable outcomes, including moderate to severe malformations in the 20th day fetuses based on osteological anomalies (Figure 2).

On the 20th day of gestation, the skulls of control albino rat fetuses were inspected, and all of their components were ossified (Figure 3). The skulls of fetuses that their mothers administered two dosages of MSG developed abnormalities' fetuses indicated a lack of cartilage production in all areas of the skull. The skull was much smaller in volume and length, with many abnormalities (Figure 3B and 3C). Modest ossification was observed in the lower jaw bones of all MSG-treated fetuses, with a progressive absence of ossification, as shown in Figure "3A and 3B" and modest ossification of the administered group's dentition.

Control fetuses' vertebral columns were well-ossified, with seven cervical,

twelve thoracic, seven lumber, four sacral, and ten caudal vertebrae.

Examination of the spinal column in fetuses maternally treated with MSG at two dosages revealed the delayed chondrification in all vertebrae, delayed ossification and chondrification in the middle of cervical vertebrae, and absence of most or all caudal vertebrae (Figure 4A).

Each pair of ribs in the control group is separated into bony and cartilaginous sternal areas. Except for the final three pairs, the sternal section of the ribs interfaces with the sternum (Figure 3A and figure 4A). There were no variations in the number of ribs or their ossification across the treatment groups. The sternum of control fetuses consists of six rod-like ossified sternebrae organized in a straight line, with the xiphisternum at the end.

Fetuses fed MSG by their moms had more or less ossifications in their sternum compared to the control group and delayed xiphoid cartilage formation (Figure 4B).

On the 20^{th} day of gestation, the pectoral girdle of the control fetuses showed a well-ossified scapula and clavicle stained with alizarin red S and a cartilaginous upper scapula stained with alcian blue. The control fetuses had a well-developed forelimb, as well as cartilaginous carpalia and metacarpalia (Figure 5).

When the MSG-treated groups were compared to the control group, the pectoral girdle and forelimb of fetuses fed MSG at various dosages revealed a reduction in size, length, and ossification level (Figure 5). At high MSG dosage, all G4 fetuses' pectoral girdles and forelimbs showed a significant lack of ossification (Figure 5). In addition, the phalanges and metacarpals of the forelimbs appeared arched in G4.

Control fetuses have a pelvic girdle made up of three bones (ilium, ischium, and pubis). Normally, the pubic symphysis is cartilaginous. The hind limbs of control fetuses include the femur, tibia, fibula, tarsals, metatarsals, and phalanges (Figure 5B). The pelvic girdle and hind limbs of MSG-treated fetuses were significantly shortened, with partial and missing ossifications of their components. Metacarpal bone and phalange cartilage drawings in fetuses from the administered group showed distortion (Figure 5B).

3.3. Effect of MSG on Fetuses' Lung Tissues

The lungs of control fetuses demonstrated the normal structure of

respiratory bronchioles and alveolar ducts (Figure 6A).

The lungs of fetuses maternally treated with different doses of MSG showed marked growth retardation with delayed development of the lung in comparison with the control one. Bronchiolar tubules are bordered by pseudostratified epithelium, which contains aberrant cells with significant nuclei disintegration (Figure 6C-F). Necrotic cells, discontinuities in various areas of the epithelium, and pyknotic dark-stained nuclei were seen (Figure 6C-F). Also, the inter-saccular septa thickened significantly, whereas the majority of the saccules narrowed. The lining epithelium of the respiratory structures is partly shed and few cells can be seen detached in their lumina (Figure 6C, E, F).

3.4. Effect of MSG on fetuses' liver tissues

The livers of control fetuses exhibited normal hepatic tissue structure, including normal central and portal regions. Hepatic tissue is made up of polyhedral hepatocytes organized radially around a central vein, divided by blood sinusoids. The portal regions are located at the edges of each lobule and include branches from the hepatic portal vein, artery, and bile duct (Figure 7A).

The livers of maternally treated fetuses with various MSG dosages were significantly damaged and showed significant deformation and disorganization, including deterioration of the hepatic lobules (Figure 7C-F). Hepatocytes were badly harmed, losing their unique appearance and exhibiting fatty changes. Necrotic cells exhibited histopathological indications such as pyknosis, karyorrhexis, and karyolysis in the nucleus. Individual hepatocytes responded well to changes. Hepatocyte destruction was seen as significant cytoplasmic vacuolization, oedema, and congestion of the central vein (Figure 7C-F), as well as lymphatic infiltration (Figure 7E).

4. DISCUSSION

Among MSG metabolites, glutamine is the most toxic and causes systemic damage **[20].** Rats have a gestation period of twenty-one days, with embryogenesis commencing on day eight and continuing for fourteen days. At this time, exposure to a teratogenic chemical can particularly trigger abnormalities of various developing organs **[21].** This investigation used MSG from the 1^{st} to 20^{th} days of pregnancy, which falls within the teratogenic window. Results

demonstrated that MSG affected rat fetuses. Pregnancy and fetal development can be negatively impacted by drug usage or chemically induced placental malfunction or injury **[22,23].**

The current investigation shows that fetuses in the MSG-treated groups were statistically significantly shorter in length compared to the normal control group. The highest decrease was seen in G2 and G4 **[20,24].** This decease may be a result of reduced substrates in the blood of mother rats administered MSG, potentially leading to insufficient nutrient supply, particularly glucose, for the fetuses. On the other hand, it was said that everyone, including children and pregnant women, can safely consume small amounts of MSG, except those who have hereditary diseases **[25,26,27,28].** In the present investigation, skeletal dysfunction and vascular abnormalities, including coagulated blood in various parts of the fetus's body, were seen in G2 and G4. Different experiments in adults showed the effect of MSG on bone and skeletal system **[29,30,31].** Compared to control animals, MSG may raise blood glucose, insulin, triglycerides, and cholesterol levels, which could contribute to potential risks to the blood and digestive system leading to cancer, heart disease, atherosclerosis, and metabolic issues **[32,27].** Skull bones show ossification retardation concerning the degree of redness, which denotes a decrease in the ossification process, particularly in G2 and G4, these findings matches with the results from a study by shosha et al. who used two different dosages of MSG 3g MSG /kg b.w and 6 g MSG /kg b.w from 1^{st} to 18^{th} days of gestation **[31].** The G1 vertebrae in the present investigation did not vary from the control. G1 which is treated with Pg.J showed a significant amelioration in body length, ossification and chondrification. Acute skeletal abnormalities in the cervical, lumbar, sacral, and caudal vertebrae were present in the fetuses in the second and fourth groups. Compared to the control group, the treated groups' sternebrae and ribs were shorter. A reduction in chondrification was shown by the cartilaginous section of the ribs' blue hue being lower than that of the control group **[33].**

Comparing the fetuses in the "G2" and "G4" groups in particular, the extent and degree of ossification had decreased in the fore girdle and limbs. Furthermore, several phalanges sustained injury, particularly in the second and fourth groups. The chondrification of the pubic symphysis, tarsals, and metatarsals varied, particularly

in G2 and G4. The hindlimb and pelvic girdle components in rats treated with MSG were smaller than in the control group **[34,35].** MSG use can promote osteoporosis, which can lead to fractures, particularly in postmenopausal and older women **[36].** The current investigation confirmed that long-term ingestion of high dosages of MSG during pregnancy can be hazardous. However, Pg.J may reduce the possible toxic effect of MSG-ingestion during pregnancy by reducing oxidative stress.

The lungs of fetuses maternally treated with various dosages of MSG revealed significant growth retardation and lung development delays. Administration of MSG during the 1^{st} to 20^{th} days of pregnancy caused developmental changes; The bronchiolar tubules are surrounded by pseudostratified epithelium and necrotic cells. The inter-saccular septa have thickened greatly, while most saccules have shrunk. Lung architecture and embryonic development were slowed, as evidenced by cell damage **[8,37,38].** The histological examination showed that some cells exhibited cytoplasmic vacuolation. Additionally, some cells desquamated inside the bronchial lumen, resulting in cellular debris. The interstitium experienced interstitial oedema due to the production of histamine by immune cells, which accelerated the infiltration process and caused protein leakage from blood capillaries **[8].** The Pg.J-treated groups showed less harm compared to MSG-treated groups.

Glutamate is absorbed in the gut by active transport system precisely for amino acids **[4].** Studies carried out by Stoll have revealed that 95% of dietary glutamate presented to the mucosa was metabolised in the first pass and that of this, 50% appeared as portal CO2, with lesser amounts as lactate and alanine.10% of dietary glutamate used for the production of proline, arginine and glutathione **[4].** linked to the production of oxidative stress by MSG, damage occurs to several organs such as the lungs and liver **[9,38].** Transverse slices of the treated fetus' liver exhibited a change in structure, with most hepatic cords no longer radially distributed around the major vein. Despite multiple toxicological examinations, MSG dosages ranging from 0.6 to 1.6 g/kg body weight altered hepatic parenchymal tissue and caused hepatocellular damage **[24,20].** Studies demonstrated that mice fed with MSG at a dose of 0.6-1.6 g/kg for 14 days resulted in enlarged livers, as well as a rise in blood levels of several liver enzymes, as oxidative stress leads to a loss in the liver's

ability to metabolize hazardous compounds **[24,20].** The hepatocytes exhibited modest to significant degenerative changes in the G2, G3, G4, and G5 treatment groups. Compared to the peripheral and intermediate zones, the hepatic lobule's centrilobular zone had fewer of these degenerative alterations. MSG treatment of adult female rats with 3 and 6 g/kg MSG from 1^{st} to 18^{th} days of gestation resulted in hepatic necrosis **[39]**, Dilatation and congestion of the hepatic sinusoids, central and portal veins **[28],** hyperplasia of bile ducts in high-dose MSG treated mothers **[40,31],** increasing lipid peroxidation (as a marker of oxidative stress) **[41].** MSG has a direct toxicological impact on hepatic cells **[31]**, as evidenced by the current investigation.

Research on MSG hasn't revealed any connection to cancer risk. However, due to potential negative health effects, experts advise against using products containing MSG. in particular, "savory" products should have a look at the contents before consuming them because they are more likely to include MSG **[42].**

According to research by Abu Elnaga *et al*., brief exposure to MSG altered both the mother and the foetus in many ways **[27].**

The current study concluded that high doses of MSG (1.6 g/kg b.w) during pregnancy adversely affected fetuses, leading to reduced body length and weight, skeletal abnormalities, and altered lungs and liver architecture by the 20^{th} day. Pg.J supplementation could mitigate oxidative damage by reducing lipid peroxidation and enhancing antioxidant enzyme activities and GSH levels.

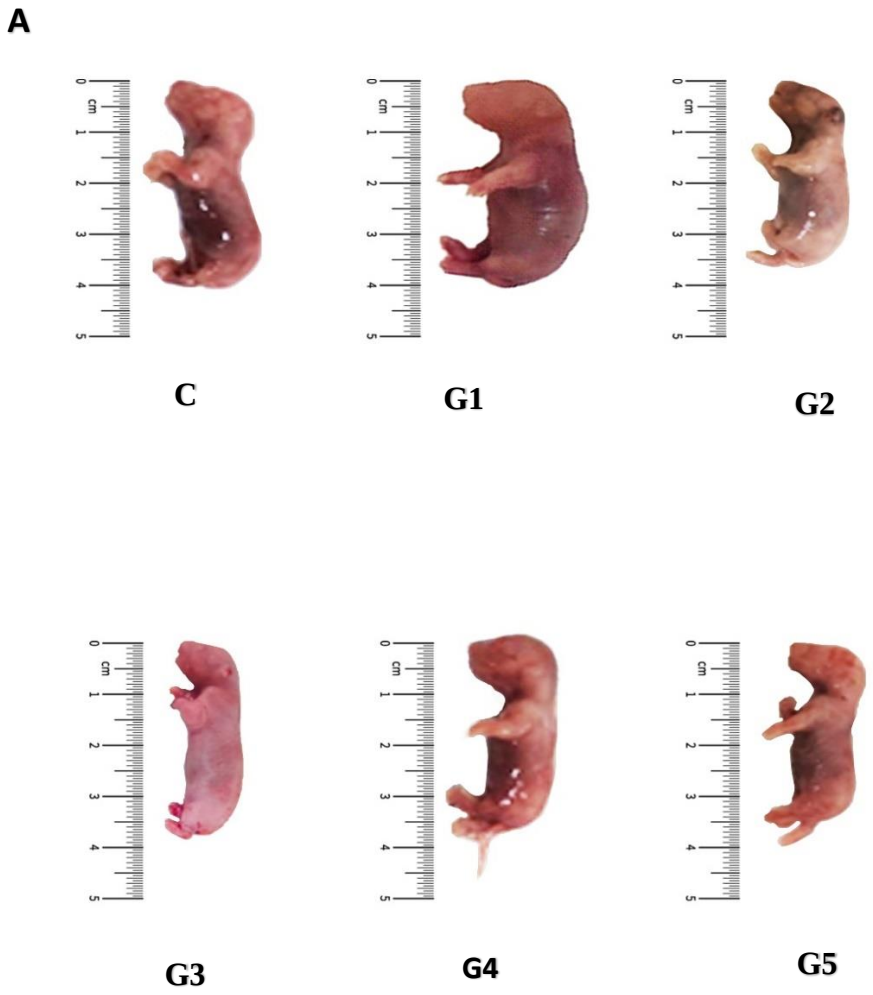

Figure 1: The effect of MSG on the morphology of fetuses.

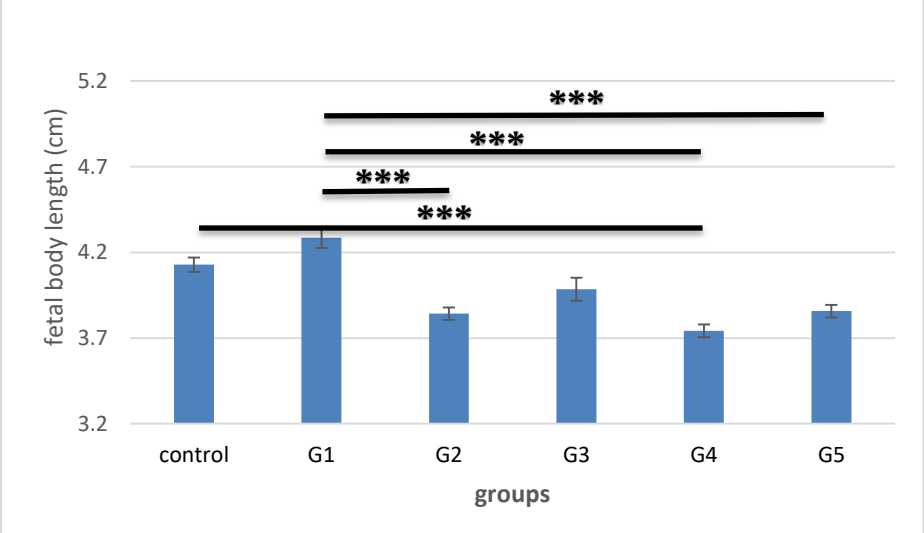

Figure 2: The fetal body length. Data are expressed as mean ± standard error. ***P<0.001.

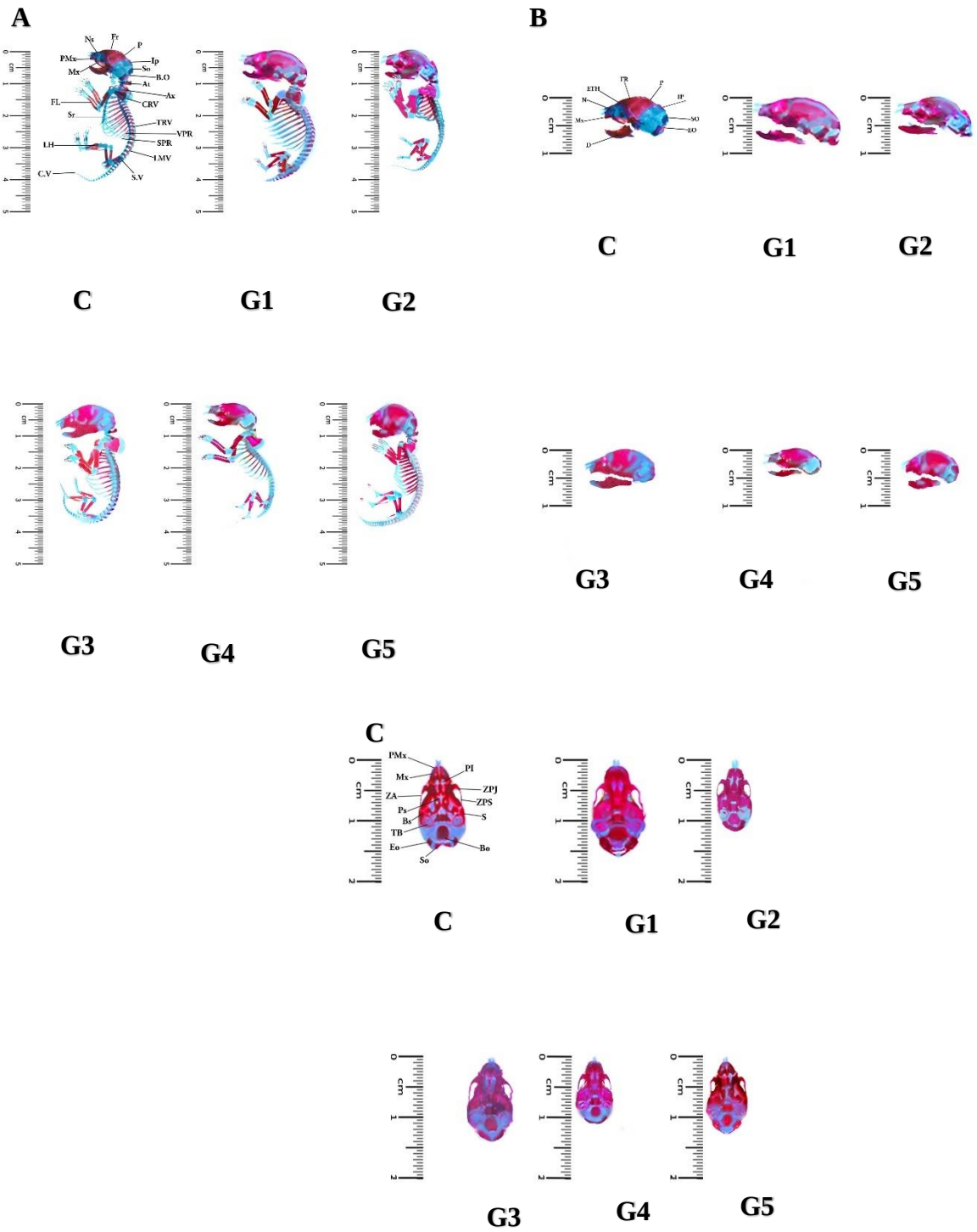

Figure 3: Lateral views on the 20th day of gestation of rat fetuses of the control and MSG-treated groups. (A) Fetal skeletal system. (B) lateral view of Fetal skull. (C) ventral view of fetal skull. The abbreviations cited in the figure were identified in Table "1".

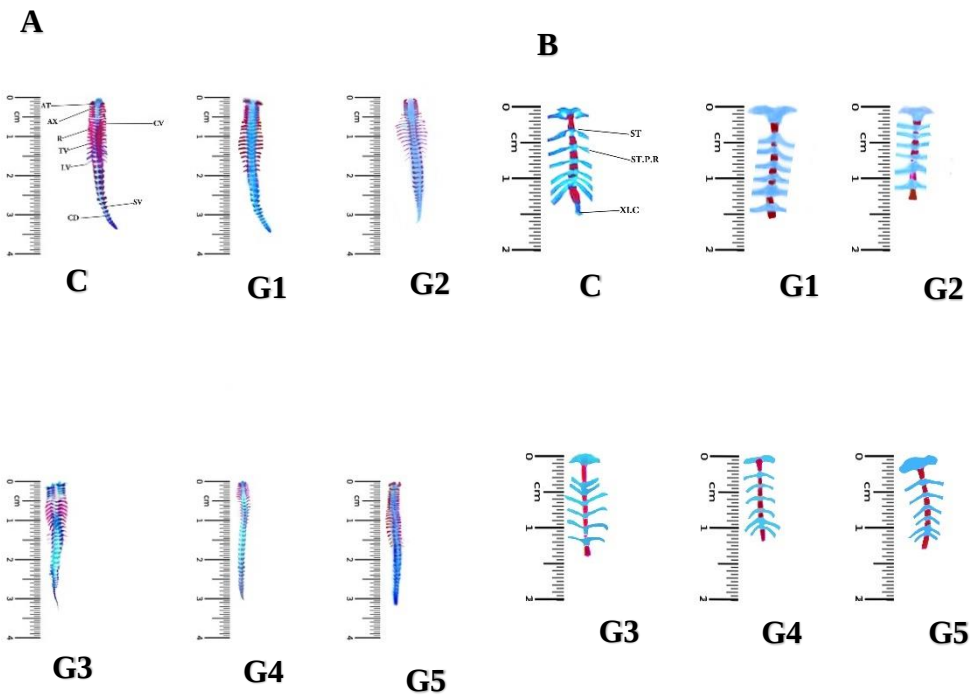

Figure 4: Ventral views on the 20th day of gestation of rats' fetuses of the control and MSG-treated groups. (A) Fetal vertebral column. (B) Fetal sternum. The abbreviations cited in the figure were identified in Table "1".

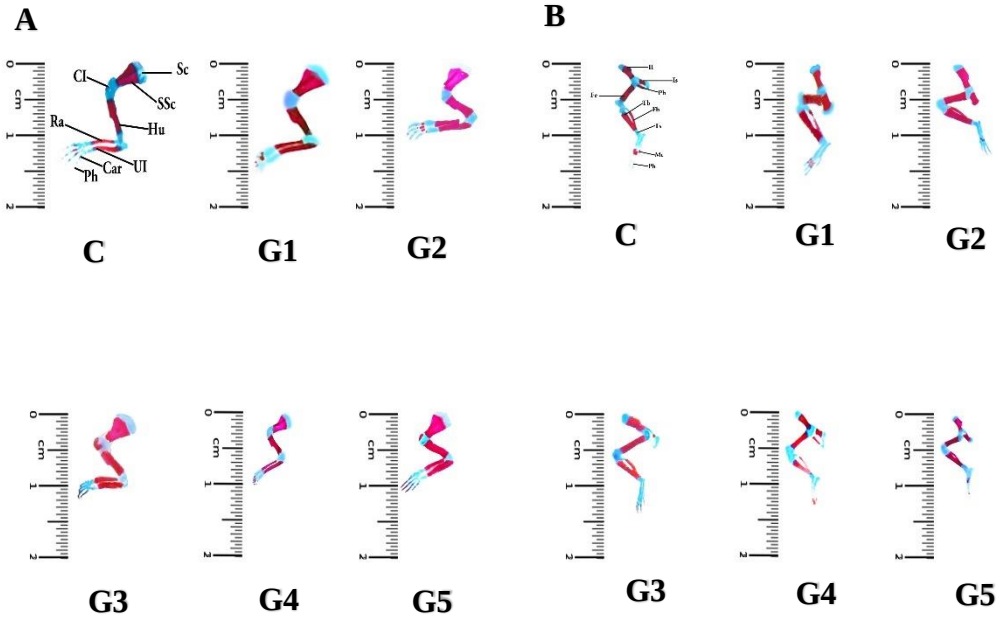

Figure 5: Lateral views on the 20th day of gestation of rat fetuses of the control and MSG-treated groups. (A) Fetal pectoral girdle and fore limb. (B) Fetal pelvic girdle and hind limb. The abbreviations cited in the figure were identified in Table "1".

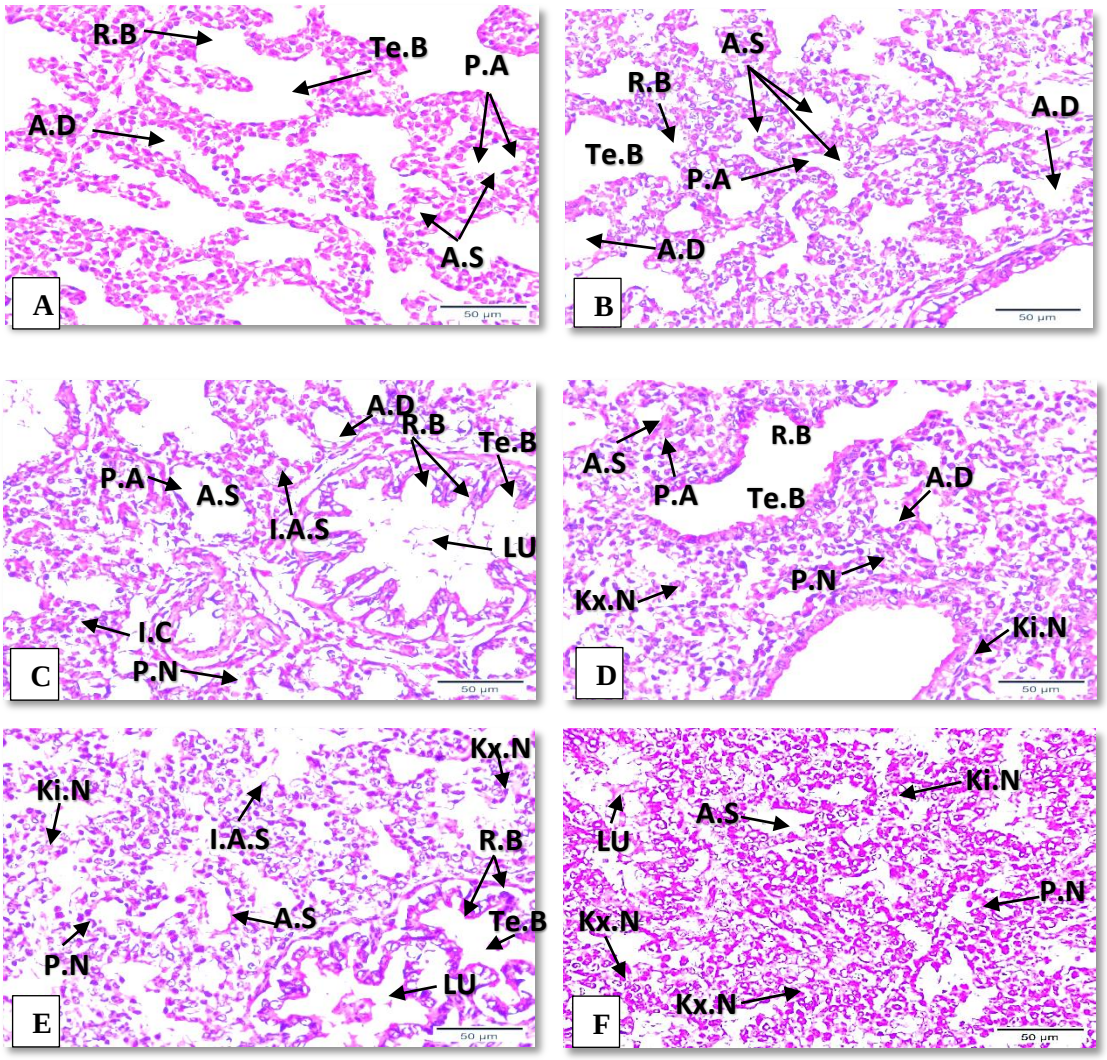

Figure 6: Photomicrograph of sections of the lungs of rats' embryos on the 20th day of pregnancy of the control and MSG-treated groups. (A) The control group. (B) Pg.J-treated group. (C-F) MSG-treated groups**.** The abbreviations cited in the figure were identified in Table "1".

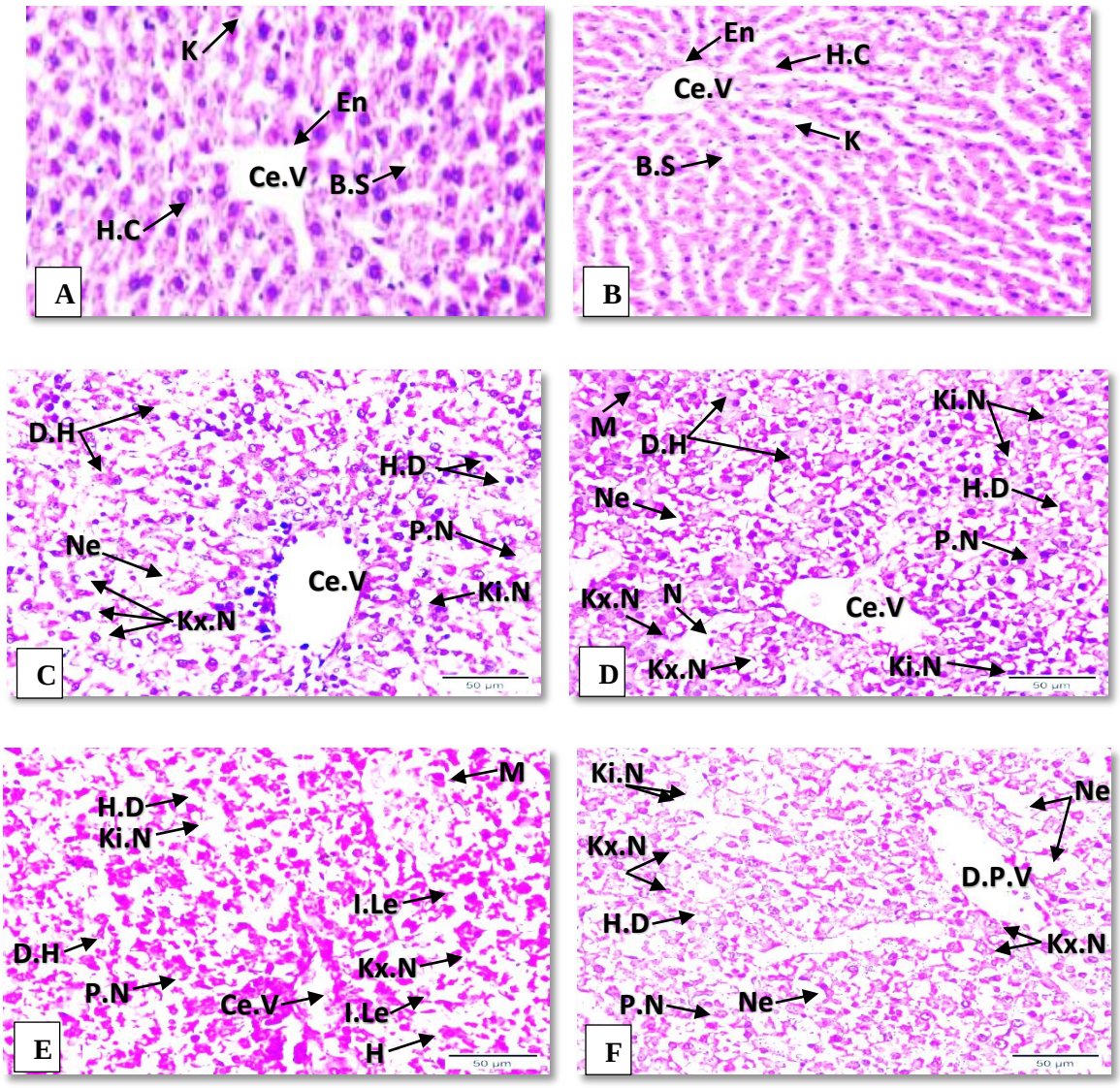

Figure 7: Photomicrograph of sections of the liver of rats' embryos on the 20th day of pregnancy of the control and MSG-treated groups. (A) The control group. (B) Pg.J-treated group. (C-F) MSG-treated groups. The abbreviations cited in the figure were identified in Table "1".

Table 1: Abbreviations cited in Figures "3-7".

Abbreviation	Bone name	Abbreviation	Bone name
A.D	Alveolar duct	LMV	Lumbar-vertebrae
A.S	Alveolar sacs	LU	Lumin
A.t	Atlas	M	Megakaryocyte
Ax	Axis	Mt	Metatarsal
B.S	Blood sinusoids	Mx	Maxilla
Bo	Basi-occipital	N	Nasal
Bs	Basi-sphenoid	Ne	Necrosis
B.S	Blood sinusoid	P	Parietal
Car	Carpales	P.A	Pulmonary alveoli
Ce.V	Central vein	Pb	Pubis
Cl	Clavicle	Ph	Phalanges
CRV	Cervical-vertebrae	Pl	palatine
C.V	Caudal-vertebrae	PMx	Pre-maxilla
D	Dentery	P.N	Pyknotic nucleus
D.H	Degeneration of hepatocyte	Ps	Pre-sphenoid
D.P.V	Dilation of hepatic portal vein	R	Ribs
En	Endothelial cells	Ra	Radius
Eo	Exo-occipital	R.B	Respiratory bronchiole
ETH	Ethmoid	Sc	Scapula
Fb	Fibula	So	Supra-occipital
Fe	Femer	SPR	Sternal-portion-ribs
FL	Fore-limb	Sq	Squamosal
FR	Frontal	Sr	Sternum
H	Hemolysis	SSc	Supra-scapula
H.C	Hepatic cells	ST	Sternebrae
H.D	Hydropic degeneration	S.V	Sacral-vertebrae
HL	Hind-limb	TB	Tympanic-bulla
Hu	Humerus	Tb	Tibia
I.A.S	Inter alveolar septa	Te.B	Terminal bronchiole
I.C	Inflammatory-cell aggregation	TRV	Thoracic-vertebrae
Il	Ilium	Ts	Tarsalia
I.Le	Leukocytic infiltration	Ul	Ulna
Ip	Interparietal	VPR	Vertebral-portion-ribs
Is	Ischium	XI.C	Xiphoid cartilage
K	Kupffer cells	Za	Zygomatic-arch
Ki.N	Karyolysed nucleus	ZPJ	Zygomatic-process jugal
Kx.N	Karyorrhexis nucleus	ZPS	Zygomatic-process-squamosal

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