Background

The liver normally consists of parenchymal cells and non-parenchymal cells. The parenchymal cells or the hepatocytes have an envelope that consists of two parallel membranes. These hepatocytes contain mitochondria (Mi) surrounded by rough endoplasmic reticulum (RER). The ribosomes (R) of these cells are free and not attached to mitochondrial membranes [1]. The non-parenchymal cells include Kupffer, satellite cells, and the endothelial cells (EC) that line the blood sinusoids (BS) [2].

Monosodium glutamate is one of the most commonly added chemicals to foods. It is the salt form of glutamic acid, a non-essential amino acid, with unique flavor-enhancing quality [3]. MSG is considered as an excitotoxin that alter normal neurotransmission functions in animal biological systems [4]. It results in harmful effects on the body organs such as brain damage, retinal degeneration, and hepatic toxicity [5]. Many studies showed that MSG can cross the placenta and reach the fetus if it was injected in pregnant animals [6]. Some studies reported that MSG has oxidative effects on various organs [7] and that it increases the risk of certain cancers [8]. The effects of food additives may be immediate or long term. Immediate effects include headache, change in energy levels, alteration in behavior, or reduced immune response. Long-term effects occur with constant exposure to the food additives and include increased risk of cancer [9]. There are many studies that dealt with the effects of MSG, but those that dealt with its effects on fetuses are few, and those dealt with the effects of its consumption before pregnancy on embryonic formation are extremely limited.

This experimental study aimed to determine the changes caused by MSG on morphology and histology of the liver of chicken embryos aged 16 days of incubation, to find out its harmful effects on liver organogenesis.
Methods
The experimental animals
Fifty chicken eggs were used in this experimental study. Their mean weight was 60–62 g. They were obtained immediately at the time of laying by the chicken and prior to their incubation from a farm in Jeddah city in Western region of Saudi Arabia. Chicken embryos were used in this experimental research as they are highly sensitive to any nutrition deficiency provided to them and they develop the congenital anomalies that produced by different drugs on mammalian embryos.

Materials
Monosodium glutamate
MSG is white crystalline powder, fast-soluble in water, supplied by the Ayaz Packaging and Food Packaging Company in Jeddah.

Experimental groups
In this study, 50 eggs were used and divided equally into two main groups. The first group (control, C) included 25 eggs, used to study the composition and normal growth of liver. The second group (MSG group) included 25 eggs treated with MSG on 0 day of incubation by making two holes inside the air sac [10]. The eggs of the both groups were incubated at 37.5°C. Liver samples were prepared for electron microscopic examination at day 16 to compare between the liver structure in the control and MSG-treated group [11, 12].

The dose used of monosodium glutamate
Each egg was injected with an effective dose of 0.1 ml of monosodium glutamate solution inside the air sac before incubation [13].

Results
The normal growth of liver (control group, C)
The liver samples of the control group at 16 days of incubation showed that the liver consists of polygonal parenchymal cells that mostly appeared irregular with 4–6 sides, and non-parenchymal cells including Kupffer cells (KC) and EC that line BS (Fig. 1C-1, C-2). According to the electronic density, the hepatic cells were divided into two types: dark hepatocytes (DC) and pale hepatocytes (PC). The DC are characterized by the presence of many cytoplasmic organelles (Fig. 1C-1, C-2). The samples of this study showed that N of hepatocytes are large and different in size from one cell to another, and that there are two types of chromatin inside them: heterochromatin (HC) and euchromatin (ECr). Each N was surrounded by nuclear envelope (NE) encrusted with holes or nuclear pores (NP) (Fig. 1C-1, C-2, C-3, C-4, C-5, C-6).

In this study, the hepatic cell was found to contain G that consist of tubular or flat membranous cisternae, with surface vesicles (V) and large gaps. These G have a convex outer face or formed face (FF) and another concave or mature face (MF). They are often located around the nucleus and alongside areas of adjacent liver cells near BC and the surface of the hepatocyte facing sinusoidal cavities (Fig. 1C-6, C-7, C-8).

The Mi within the hepatic cells of this study samples appeared as multiform (circular, oval-to-long), multivesicular bodies and moderately dense organelles, which have two membranes separated by a space. Their outer membrane is semi-permeable and contains enzymes, while the inner membrane is folded inwards forming cisternae. Their inner cavity is filled with the mitochondrial matrix. A close spatial correlation between Mi and coarse endoplasmic network was noted (Fig. 1C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8).

This study showed that the cytoplasm contains cellular organelles, which included Mi, G, RER, SER, LY1, LY2, multivesicular bodies, and multivesicular bodies (MB), and peroxisomes (P) (Fig. 1C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8).

The studied sample showed that the surface of hepatocytes is divided into three parts: the first part faces the bile ducts (BC), the second part faces the adjacent hepatic cells, and the third part faces Disse space (DS). Also, there were desmosomes (D) and tight junction (TG) between cells (Fig. 1C-7, C-8, C-9).

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Fig. 1 Control (C): C 1–9. The normal structure of liver cells in chicken embryos. They show different types of the cells, N, Mi, Type of LY, G, RER, SER, LD, BC and BS.
The effect of monosodium glutamate on the structure of the liver of chicken embryos (MSG group)

Examination of samples from the liver aged 16 days of MSG group by electron microscope showed many structural changes and histopathological degradation of liver cells including changes in N such as irregular NE, dilatation of NP, or increased HC. Also, many N have lost their electron density, and others showed marked derangement of NE either partially or totally. Moreover, there was a space between N and cytoplasm in some places called the nuclear space, or there was a dilation between the two layers of NE. Many N were seen at cells edges (nuclear margination) (Fig. 2 T-3, T-4).

In this study, Mi appeared to have irregular envelope with abnormal cristae and high electronic density due to derangement of its components (mitochondrial envelope, cristae, and mitochondrial matrix). Some Mi appeared as dark or decaying bodies with difficult differentiation of their internal contents (Figs. 2 T-3, T-5, T-6 and 3 T-7).

This study samples also showed disruption and disorganization of RER, where they lost their parallel cisternae and showed fragmentation, vacuolation, dilation, or degradation in some parts (Fig. 2 T-3, T-4, T-6).

The SER observed in this study samples showed proliferation of its units and disturbance of its distribution. Also, there was a spatial correlation between SER and GL (Figs. 2 T-5, T-6 and 3 T-8, T-12).

In the current study, it was difficult to see G in many of the treated cells, and even if it was seen, it appeared as either atrophic or hypertrophied bodies with expanded or vacuolated membranes (Figs. 2 T-5 and 3 T-9).

It was observed also in this study samples that there is variable distribution of LY1 and LY2 as well as P inside the treated cells. The P appeared as clusters, and some of which contained reaction products. Also, LY2 appeared attached to LD (Figs. 2 T-6 and 3 T-9).

The results of this study showed that BC appeared as diluted areas with short MV, while there was expansion and increase in the number of desmosomal junctions surrounding multiplied BC (Figs. 2 T-5, T-6 and 3 T-10).

In this study, BS were extensive with destruction of their walls and alteration of lining cells, EC and KC. The sinusoidal space was filled with RBCs, cellular residues, and collagen fibers (Figs. 2 T-1 and 3 T-11).

It was observed that there is excessive fatty infiltration in the cells lining BS, where LD were often related to LY2 and they were either separate or merged with each other. These LD variable in size and there was increase in their number within MSG treated liver cells (Figs. 2 T-1, T-2, T-6 and 3 T-10, T-12).

This study showed several collagen fibers in the connective tissues and in between liver cells; however, the GL particles were few (Figs. 2 T-5, T-6 and 3 T-8, T-12).

In this study, there was expansion of the intercellular (IS) spaces and it was associated with expansion of BS, which indicates that the first response to cellular damage was cell expansion (Figs. 2 T-2, T-4 and 3 T-11).

Discussion

The normal growth of liver (control group, C)

This study revealed the general structure of the liver, which is formed of DC and PC. The DC are characterized by the presence of many cytoplasmic organelles and relatively variable nuclei (N) with dark chromatin. However, PC contain more abundant RER and free R and their N are true with equally distributed chromatin.

This agrees with Bourne [14] who also described that the liver contains KC, EC that line BS, and parenchymal cells which are divided into DC and PC. Also, Medlock and Haar [15] and Abdel-Fatah [16] stated that cytoplasm of DC contains large number of Mi, RER, primary lysosomes (LY1), secondary lysosomes (LY2), lipid droplet (LD), and Golgi apparatus (G), with less smooth endoplasmic reticulum (SER).

The N of hepatocytes are large, surrounded by NE, and contain two types of nuclear chromatin (ECr and HC). In agreement with our results, Abdel-Fatah [16] and Bruni and Porter [17] reported that N contain two types of chromatin, HC and ECr, and that they are surrounded by double layered nuclear membrane that contains many NP spread over variable distances.

All the cytoplasmic organelles appeared in the liver cells of our study including RER and R. This is consistent with Moule [18] who recorded that the liver cells at the age of 8 days of incubation showed RER and R which are scattered in the cytoplasm. Bruni and Porter [17] reported that there is difference in the position of endoplasmic reticulum (ER) not only from one liver cell to another, but also in a single liver cell, which indicated that ER is not a standard compound, but there may be changes in its direction, position, or composition. They also showed that SER are distributed in areas of the cell rich in glycogen (GL).

The G appeared to have two faces (FF and MF) and contain. It was located around the N, near BC and the BS. This description was corroborated by Abdel-Fatah [16], Johnson [19], Al-Yousuf [20], and Burkitt et al. [21] who described G as a group of cisternae and parallel V with many gaps, and that they have two sides, convex and concave, and lie very close to the N, BC, and BS.

The Mi appeared with variable shapes and sizes. It had two membranes separated by a space, where the inner membrane was folded inwards to form cisternae. It was spatially associated with the RER. This is consistent with Bruni and Porter [17] who reported that Mi are closely related to RER. Al-Yousuf [20] and Burkitt et al. [21] added that Mi are variable in size but mostly rectangular, and their number may reach up to 2000 per cell.
The lysosomes appeared in their various types which include Ly1, Ly2, P, and MB. This agrees with Bruni and Porter [17] and Burkitt et al. [21] who reported that LY1 appeared as membrane-bounded organelles, variable in size and shape, and contained undifferentiated granular materials, while LY2 were more variable in their...
appearance and some of them were very dense. Moreover, P appeared as small spherical membrane bounded structures.

Glycogen particles were seen collected in the cytoplasm near SER. Also, Han and Holmsted [22] noted that SER are closely related to GL particles. However, these particles are rare or completely absent in areas of RER and/or R.

LD of variable sizes were detected in the liver cells of this study. This agreed with Abdel-Fatah [16] and Mahmoud [23] who described LD as multi-form fatty globuli of variable sizes.

BC regions were seen between adjacent cells. The plasma membranes of the cells close to BC were bound by tight bonds which include (ZO), (ZA), and D junctions. This result was supported by Han and Holmsted [22] who reported that BC are areas between the membranes of adjacent hepatocytes.

In this study samples, the hepatic cells were separated by BS lined with EC and KC. The EC lining BS were separated from liver cells by a space called DS. Bruni and Porter [17] explained that blood fluids pass freely through EC of BS to DS to be in direct contact with the

Fig. 3 T 7–12. The liver of chicken embryos, treated with MSG (MSG group), at the age of 16 days. It shows the structural changes of liver cells. Also, it shows widening of inter-cellular spaces and deformation of BC and BS.
hepatic cells facilitating the exchange of important substances between blood and hepatic cells.

The effect of monosodium glutamate on the structure of the liver of chicken embryos (MSG group)

Many structural changes had occurred in the liver cells after exposure to MSG. The changes that occurred in N included irregular NE, dilatation of NP, or increased HC. Moreover, many N have lost their electron density. This agree with Farhoud [24] and Abdel-Fatah [16] who reported that the changes occurred in N of liver cells of chicken embryos included increase in HC amount and disturbance of its distribution. Also, the NE became irregular and interrupted with expansion of space between its two layers and dilatation of NP with some connection between nuclear material and cytoplasm.

Major changes have occurred in mitochondria where their envelope became irregular and their components get degraded. These results are supported by Abdel-Fatah [16] who reported appearance of abnormal Mi, which were either decayed, partially or completely atrophied with disruption of its components, increase of its density, and deposition of decayed materials inside it. Hummmdi [25] indicated that the marked changes that occurred in Mi, including its atrophy or deposition of decayed materials inside it, resulted in loss of its functions and damage of the cell.

The RER showed fragmentation, vacuolation, and dilatation in some of its parts. This agrees with the results of Abdel-Fatah [16] who confirmed that one of the most prominent observations on RER was its appearance either fragmented or vacuolated with disturbances in its shape. Also, it sometimes appeared decayed and free of R.

SER showed proliferation of its units and disturbance of its distribution. The proliferation of SER might be explained by its ability to detoxify MSG. This is consistent with Moody and Reddy [26] and Ahmed [27] who reported a close association between the proliferation of SER and the degree of cell damage. Ayman et al. [28] explained the increase in SER proliferation as a rapid response to the adverse effects on cells and that it is associated with an increase in enzymes’ activity to increase the cells ability to detoxify harmful substances.

The G appeared as either atrophic or hypertrophied bodies with expanded or vacuolated membranes.

This can be explained by that G are organelles characterized by its rapid decay and that they are difficult to be detected. This observation was supported by Abdel-Fatah [16] who reported that G were difficult to be distinguished. They were found inside hepatic cell in the form of either atrophied or hypertrophied bodies and often appeared near the nucleus or the edges of hepatic cell adjacent to BC.

Our samples showed variable distribution of LY1, LY2, and P inside the treated cells, and some of them appeared attached to LD or contained reactions products. This agrees with Ahmed [27] and de Duve [29] who reported increase in LY numbers and hydrolysis with release of LY enzymes such as phosphatase secondary to cell injury.

BC appeared as dilated areas with short MV. Also, there was decrease in their numbers and increase in the number of desmosomal junctions surrounding the multiplied BC. This is supported by Abdel-Fatah [16] results which showed that during acute injury of hepatic cells, the D appeared wide, proliferated with distortion of their composition.

BS were extensive with destruction of their walls and enlargement of their lining cells. The BS cavity appeared filled with RBCs, cellular residues, and collagen fibers. This agrees with the observations of Hassan [30] and Cotran [31] who attributed the enlargement of KC to defensive activity of these cells against poisoning. Abdel-Fatah [16] and Eid et al. [8] emphasized that BS appeared dilated and more extended with presence of many cellular residues, collagen fibers, broken MV, and dead cells inside their cavities.

There was variable sized cytoplasmic vacuoles in MSG-treated liver cells as reported also by Pfeifer and Bannasch [32], Tuchweber et al. [33], and Abbasi et al. [34]. These vacuoles may be caused by mitochondrial swelling or decomposition of mitochondrial remnants as reported by Takano et al. [35], or due to enzyme digestion of cell organelles due to cell injury as reported by Cotran [31], Biondo-Simões et al. [36], Aldana et al. [37], and Luty et al. [38]. Also, Bourne [14] reported that formation of these vacuoles may be a part of defensive mechanism to prevent interference with cell vital activities or may be due to sinusoidal dilatation as reported by Shibayama et al. [39]. The membrane surrounding these vacuoles showed the same enzymatic activity of liver cell membrane; so many scientists believe that they are indentations from cell membrane. Tuchweber et al. [33], Takano et al. [35], and Burkitt [40] reported that these vacuoles are LD that accumulated secondary to obstruction of fatty acids metabolism by liver cells.

There were excessive collagen fibers in connective tissues and between liver cells of our samples. This agrees with Farhoud [24] who reported large amount of collagen fibers in the base plate of the mouse at 3rd week of MSG injection. Awad [41] added that the amount of collagen fibers in connective tissues barriers and among cells was more or less than control sample on days 10–12 of preparation, and as growth advanced at 15–18 days of preparation, there was an increase in the amount of collagen fibers in barriers between cells.
Also there were a few GL particles in the MSG-treated cells and this was also observed by Dixon et al. [42] who found depletion of GL from cells.

There was expansion in the intercellular (IS) spaces associated with expansion of BS. This was confirmed by Young [43] who reported that intercellular expansion is a common phenomenon associated with BS expansion and resulted from fluids accumulation between cellular components as collagen fibers.

The changes that occur in the cellular functions as a result of the toxic compounds include changes in cell membrane permeability and affection of movement of materials to and from the cells. They also include changes in cellular enzymatic activities, changes in cellular division rates, changes in DNA and protein synthesis, changes in cell respiratory processes rate, and changes in energy molecules availability. Al-Ghamdi [44] reported that the harmful effects of the toxic chemicals may be caused by binding of these toxic chemicals to the biomolecules. This binding may be reversible or irreversible leading to apoptosis or affection of the cellular structure.

The effects of MSG on liver tissues can be explained by its ability to infiltrate and cross blood-brain-barrier due to incomplete development and weakness of this barrier, especially in fetuses, newborns and infants, causing high toxicity followed by defects in growth and differentiation of various tissues and cells. It also induces neurotoxicity by interacting with N-methyl-D-aspartate (NMDA) receptors causing inhibition of membrane proteins formation and change of the cell membrane organization causing cell destruction.

Burkitt et al. [21] explained that cellular decomposition occurs secondary to the inflammatory reactions of body’s immune system against damage caused by chemical compounds, parasitic and viral infections, and harmful substances.

Overstreet et al. [45] concluded that MSG had ability to cross epithelial cells membranes in peritoneal cavity and break down into sodium and glutamic acid where some of glutamic acid is excreted and the rest is converted into glutamate and during this process, the liver cells try to repair the damage occurred in their organelles. With the high percentage of glutamate, the liver cells cannot excrete or detoxify it, so a series of hemolytic changes occurs in liver gradually and finally death of the liver cells occur. Anbarkeh et al. [7] reported that cytotoxicity (apoptosis) may occur due to many factors as DNA damage, loss of survival signals, or oxidative voltage. Giorgio et al. [46] said that apoptosis occurred when the cell was severely injured as cell swells and ruptures and these effects occur because the injury prevents the cell from adjusting balance of its fluid and ions that are usually pumped out of the cell but in case of infection, they flow into cells.

Anindita et al. [47] and Hassan et al. [48] reported that MSG administration resulted in significant changes in apoptotic biomarkers as programmed cell death protein-1 related to liver damage and decrease in hepatic cell thickness.

Conclusions
These results suggest that MSG plays a role in cellular oxidative stress, which in turn lead to cell death, increased apoptosis, DNA and RNA destructions, and lack of protein production which is necessary for receptors formation. Lack of cells thickness and disturbance of the rate of their division and proliferation caused delayed growth and disruption of liver formation in general.

Abbreviations
BC: Bile ducts; BS: Blood sinusoids; DC: Dark hepatocytes; DS: Disse space; EC: Endothelial cells; ECR: Euchromatin; ER: Endoplasmic reticulum; FF: Formed face; G: Golgi apparatus; GL: Glycogen; HC: Heterochromatin; KC: Kupffer cells; LD: Lipid droplets; LY: Lysosomes; LY1: Primary lysosomes; LY2: Secondary lysosomes; MB: Multivesicular bodies; MF: Mature face; M: Mitochondria; MSG: Monosodium glutamate; MV: Microvilli; N: Nuclei; NE: Nuclear envelope; NP: Nuclear pores; P: Peroxisomes; PC: Pale hepatocytes; R: Ribosomes; RER: Rough endoplasmic reticulum; SER: Smooth endoplasmic reticulum; TG: Tight junction; V: Vesicles; ZA: Zone of attachment; ZO: Zone of obstruction

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Author's contributions
FAA made the design of the work, the conception of the idea, acquisition, analysis and interpretation of data, and finally the revision and final approval of the manuscript.

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References
1. Ward DB, Pollak J (1967) The phospholipid composition of embryonic chick liver microsomes. Biochem J 104(3):861–865. https://doi.org/10.1042/bj1040861
2. Kwak KA, Cho HJ, Yang JY, Park YS (2018) Current perspectives regarding stem cell-based therapy for liver cirrhosis. Can J Gastroenterol 2018;41:97857
3. Quines CB, Rosa SG, Da Rocha JT, Gai BM, Botolatto CF, Duarte MM, Nogueira CW (2014) Monosodium glutamate, a food additive, induces depressive-like and anxiogenic-like behaviors in young rats. Life Sci J 107(1-2):27–31. https://doi.org/10.1016/j.lfs.2014.04.032
4. Mahaliyana A, Fasmina M, Alahakoon A, Wickrama G (2016) Toxicity effects of monosodium glutamate (MSG) on embryonic development of zebrafish
Al-Ghamdi Egyptian Liver Journal (2021) 11:38

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