Effect of Monosodium Glutamate on Chick Embryo Development

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Abstract: Monosodium glutamate (MSG) is a natural neurotransmitter amino acid and a flavoring agent added to many processed food and used by many housewives in cooking. Fertile chicken eggs were injected once with (0.75mg MSG/gm. egg weight) in the air chamber before incubation. Eggs were then incubated under normal incubation conditions. Embryos were extracted on day 7, 10 and 14 of incubation. Treated embryos showed different congenital malformations such as growth retardation and subcutaneous bleeding in 7, 10 and 14 days compared to the controls. Abdominal hernia was seen in 7 and 10 day treated embryos. Most of the congenital malformations were seen in 10 day treated chick embryos such as brain deformation, monophthalmia and beak malformation. Histological study of the developing liver in the studied ages of the treated embryos showed that liver seemed to have less cell density and a dilation of venous canals and blood sinusoid. Fibrosis, bleeding, hemorrhage and congestion were seen in the central and portal veins. Many cavities appeared in the peripheral liver parts compared to controls. On the cellular level many cells had a granular appearance. Also an increase in the number and size of lipid droplets was seen in the treated sections compared to the controls. pyknotic, karyolisis were seen in treated hepatocytes Also phagocytic cells were seen in blood sinusoids. Necrosis was also seen in treated sections. It was concluded that a single low dose of monosodium glutamate might affect chick embryonic development causing growth retardation, congenital malformations, and liver degeneration.
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Key words: chick embryo, monosodium glutamate, liver, growth retardation, monophthalmia.

1. Introduction

Monosodium glutamate (MSG) is a natural excitatory neurotransmitter in brain (Bhattacharya *et al*., 2011). The artificial form of MSG is used as a food additive and flavoring agent (E621). Many studies highlighted the adverse effects of MSG when consumed. Eye and retina tissue was severely affected when chick embryos were treated with monosodium glutamate **(Al-Jahdali and Al-Qudsi 2012).** Liver tissue was affected in neonate mice injected with 2mg/gm body weight MSG **(Bhattacharya *et al*., 2011).** Also high levels of MSG added to the food of broiler chicks caused adverse effects on the nervous tissue **(Ati *et al*., 2009)**. Recent studies related between MSG and obesity **(Tawfik and Al-Badr 2012),** and pathological liver changes **(Farr *et al*., 2010)**. Also studies showed that establishing mice on a regular diet containing MSG can cause hepatic microsteatosis in their offspring **(Collison *et al*., 2009)**. Moreover it was shown that high levels of glutamate might be related directly or indirectly with the loss of β-cells **(Davalli *et al*., 2012).**

The liver is known to be the organ responsible for deamination. Many studies showed that MSG affected liver in adult **(Farr *et al*., 2010)** or neonate mice **(Bhattacharya, 2011).** Therefore it was important to see to what extent the liver development was affected by MSG. chick embryo has been an important animal model in the field of embryology and developmental biology, as its developing features are very well documented, which makes it easy for the researcher to compare the control finding in his study to previous studies detecting any changes due to improper incubation or other factors, making sure that any changes seen in the treated embryos were the cause of the treatment.

The objective of this research was to study the effect of one low dose of MSG (0.75mg MSG/gm. egg weight) on chick embryo development measuring growth parameters and examining liver tissue throughout its development.

2. Materials and methods

This study was given approval for the methodology and other ethical issues concerning the work by Biology Department, King Abdulaziz University.

Chemicals:

Monosodium Glutamate (MSG) was purchased as powder from Al-Mizani medical corporation – Saudi Arabia. MSG solution was made by dissolving 30 mg of MSG into 0.1 ml of distilled water.

Experimental design:

Fertile chicken eggs (n = 60) (average weight 40 gm) were collected fresh from a private farm in Thual (North of Jeddah city in Saudi Arabia). Fertilized chicken eggs were divided into two main groups;

control and treated. The treated group was injected once with 0.1 ml of the MSG solution (0.75mg MSG/gm. egg weight) in the air chamber before incubation. Similarly the control group was injected with 0.1 ml of distilled water. Injection of eggs was done according to the method described by **Allam *et al*., 1976** Eggs were incubated in a special rotating incubator.

Specimen collection and photography

On day 7, 10 and 14 the eggs were opened, the embryos were extracted, cleaned by washing with saline solution. Embryos were then patted dry and weighed. Then all embryos were photographed. 10 and 14 day embryos were dissected to extract the liver. 7 day whole **e**mbryos and the livers of 10 and 14 day embryos were fixed in formalin 10%.

Histological study:

Specimens were processed for histological sections and embedded in paraffin wax. Wax blocks were cut at 5µ, and then stained with haematoxylin and eosin.

Photographing:

All embryos were photographed using a Sony corp. digital still camera model no. DSC-T100SKD; a ruler was put near the embryo to be used as a scale when performing morphometric using the photos. The camera zooming and distance between the camera and specimen were the same for all whole body photos. Embryos were also photographed using an Olympus SZ61 dissecting microscope connected to a Leica DFC280 camera

Histological sections were photographed using an Olympus SZx10 stereo microscope with DPZ-BSW camera for a general view of liver position according to other body organs in 7 day embryos. They were also photographed using a compound microscope Nikon eclipse E400 connected to a digital camera (Nikon Y-IDP).

Morphometric studies:

Measurements of all control and treated specimens were taken from the photographs. The measurements taken were full embryonic length, neck length, beak length, back head width, using a computer program "Image tool" (http://ddsdx.uthscsa.edu/dig/itdesc). (See Figure1). All readings were saved in Excel 2003.

Statistical analysis:

Data was analyzed using SPSS 13. The test used with normal distribution was Anova, Student-neuman Keul test. In case of abnormal distribution Man-Whiteney U test was used from the non-parametric test. Significance was at $p<0.05$.

3. Results

Congenital malformations

Intense bleeding was seen when opening eggs to collect embryos. Growth retardation and subcutaneous bleeding were seen in several treated chick embryos of 7, 10 and 14 days compared to the controls. Abdominal hernia was seen in 7 and 10 day treated embryos (Figure 2). Other congenital malformations were seen in 10 day treated chick embryos such as brain and beak deformation and monophthalmia (Figure 3).

Body growth parameters

A non-significant decrease was seen in whole body weight and whole body length of treated 7,10 and 14 day chick embryos compared to controls.MSG caused a decrease in neck length in 7,10 and 14 day treated chick embryos compared to the controls which was only significant on day 10 and 14 (P=0.009, P=0.001) respectively. A significant decrease was seen in the beak length of 7,10 and 14 day treated embryos compared to the controls (P=0.029, P= 0.009, P= 0.011) respectively. (Figure 4).

Effects on liver development

General view of cross sections of abdominal liver area of 7 day treated chick embryos showed that the liver was in the same region as the controls; however it seemed to have less cell density and a dilation of venous canals and blood sinusoid was seen as well as bleeding. The liver appeared surrounded by a connective tissue lymphatic capsule (C) and formed from non-distinguished hepatic cords (HC) compared to the control sections where hepatic cords were very clear.

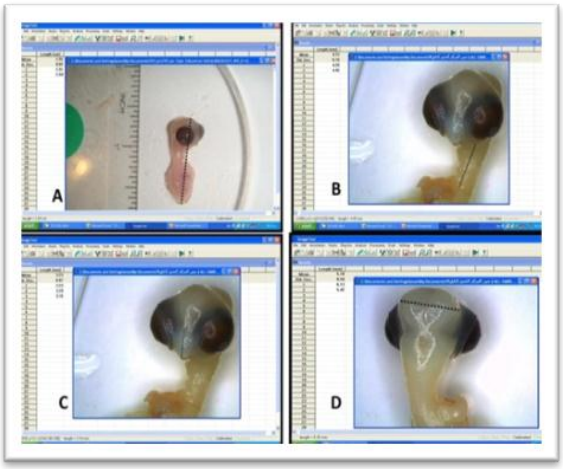

Figure 1: Showing method of measuring
(A) Full body length,
(B) Neck length,
(C) Beak length,
(D) Back head width using image tool program.

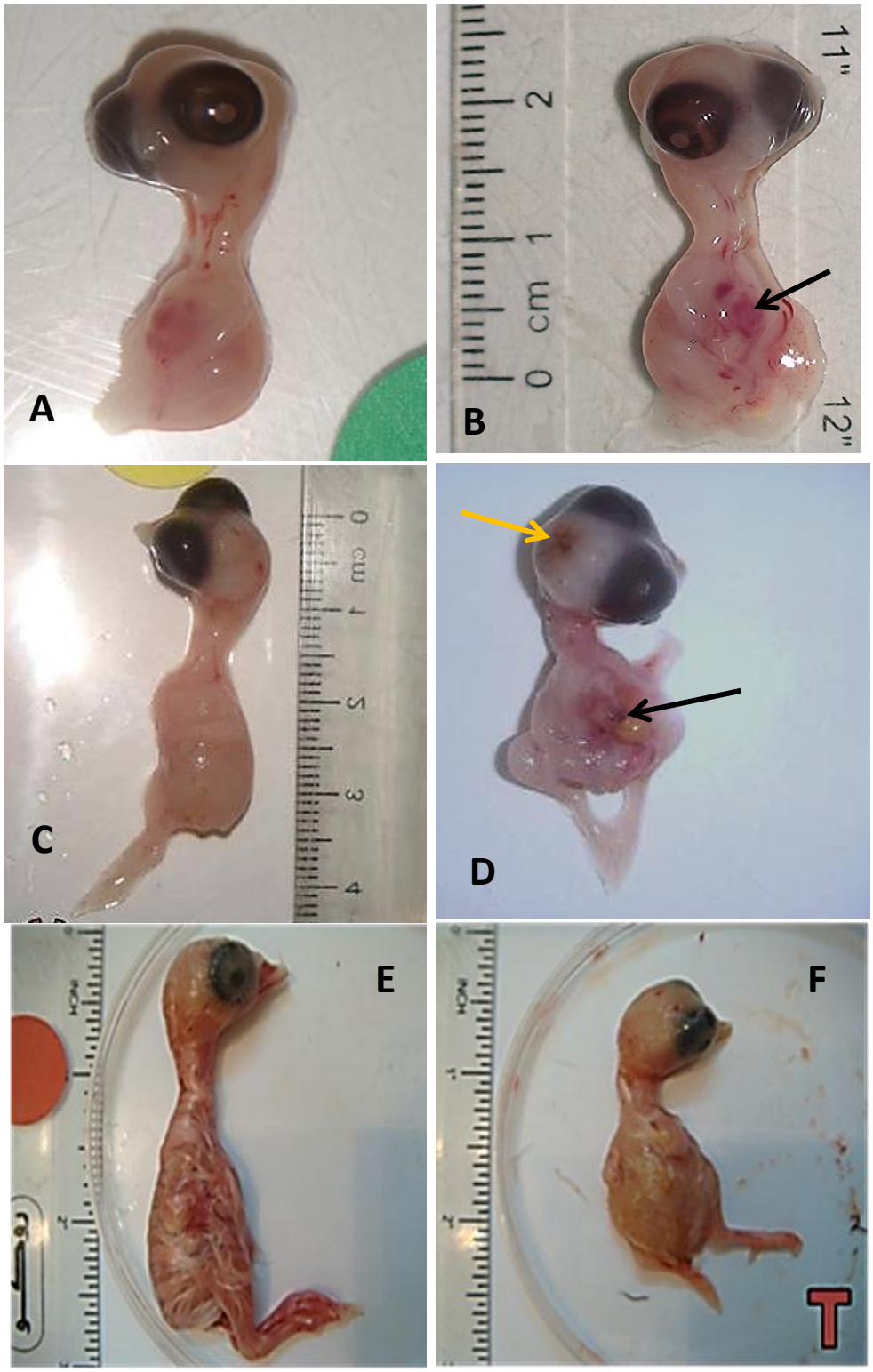

Figure 2 showing some congenital malformations caused by MSG in chick embryos. (A) control 7 day chick embryo, (B) treated 7 day chick embryo note the abdominal hernia (black arrow). (C) control 10 day embryos, (D) treated 10 day embryo note the deformed shape of the embryo, the abdominal hernia (black arrow) and brain bleeding (yellow arrow). (E) Control 14 day chick embryo, (F) treated 14 day chick embryo note the smaller size of the treated embryo.

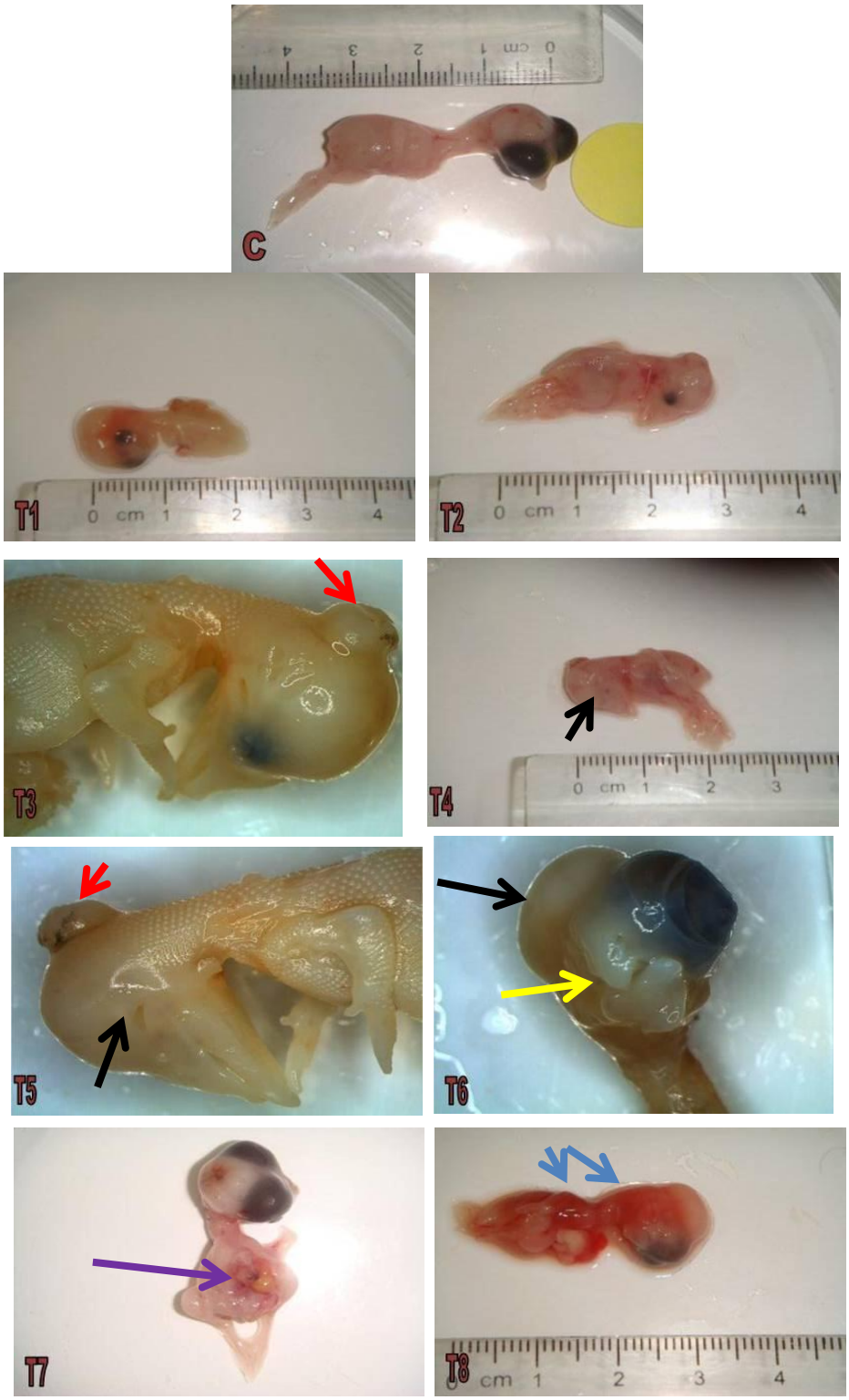

Figure 3 showing some congenital malformations caused by MSG in 10 days chick embryos. (C) control 10 day chick embryo. (T1, T2, T3, T4, T5, T6, T7, T8) 10 day treated chick embryos with different congenital malformations. Note the small size of embryo in T1, small eye size in T2 and T3, Monophthlmia in,T4, T5 and T6 (black arrow), brain malformation in T3 and T5 (red arrow), beak malformation in T6 (yellow arrow), abdominal hernia in T7 (purple arrow), heavy bleeding in T8 (blue arrows)

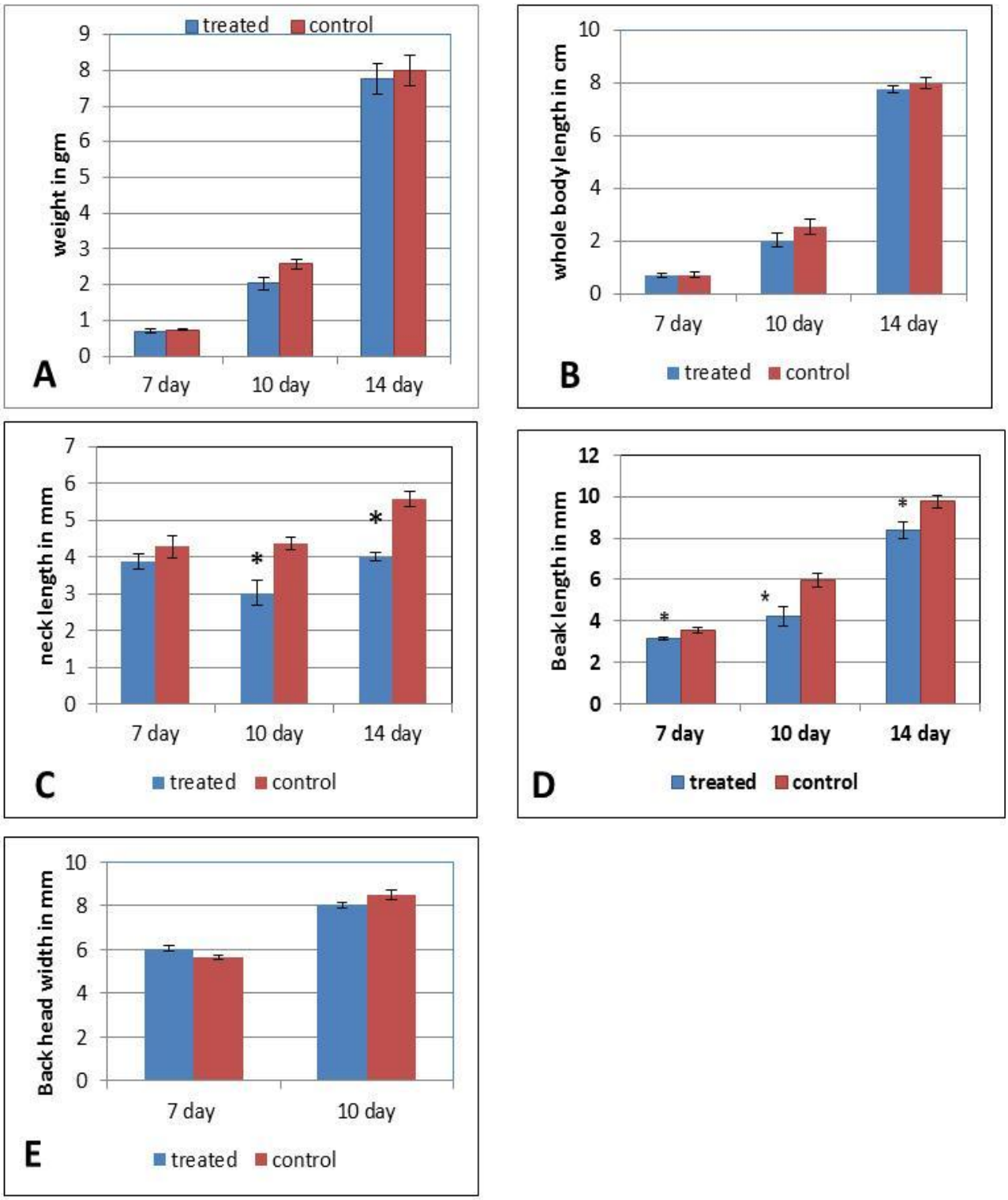

Figure 4 graphs showing the effect of MSG on chick embryos morphometric measurements. (A) whole body weight, (B) whole body length, (C) neck length, (D) beak length, (E) back head width. . Values are means ±SE, taken from 10 samples. For control and each treatment (*) $p< 0.05$

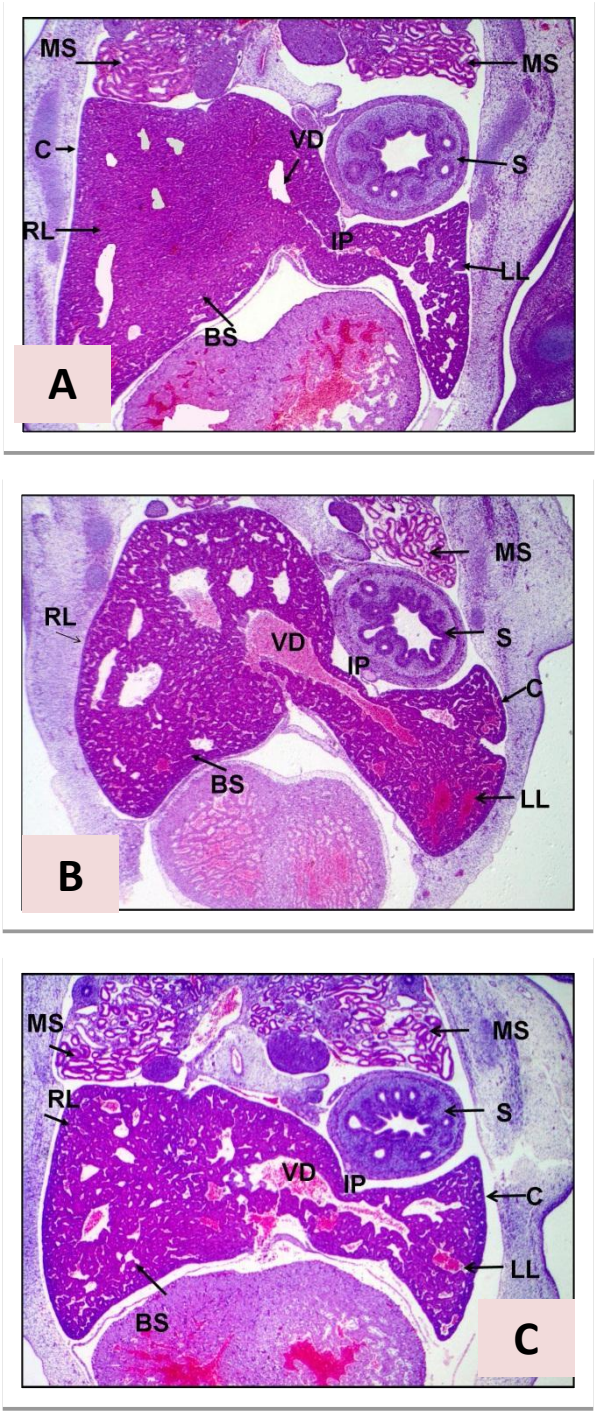

Figure 5 showing micrographs of the abdominal area of 7 day chick embryo (A) control (B and C) treated.
KEY: (RL) Right Lobe of Liver (LL) Left Lobe of Liver (IP) Intermediate Portion C) Connective Tissue Capsule (MS) Mesonephros (S) Stomach (VD) Venous Ducts (BS) Blood Sinusoids. Note the venous ducts (VD) filled with red blood cells in section (B and C).
H&E 40X

Histological cross sections of 7 day treated chick embryos showed a clear hemorrhage and congestion in the central vein also fibrosis was seen as the inner central vein lining had increased thickness and had a deposit of soft collagen fibers. Around the central vein many damaged cells were noticed as a result of central vein lining epithelial cells detachment. (Figs. 3-27B,3-28B).

Histological liver sections of 10 days MSG treated embryos showed that the liver connective tissue capsule (C) seemed thick and detached compared to the controls. Also the hepatic cords (HC) seemed less dense and undifferentiated compared to the controls. Blood sinusoids (BS) appeared to have a wider lumen with congestion and bleeding compared to the controls. The portal vein appeared congested and had fibrosis in its wall.

Histological examination of liver cross sections of 14 day MSG treated chick embryos and comparing them to the controls showed that hepatic tissue was less dense. Hepatic veins (HV) seemed wider and filled with blood cells. Hepatic cords (HC) were bilateral around the central vein (CV) as in controls. Hemorrhage and congestion were seen in the central vein and wider blood sinusoids. Many cavities appeared in the peripheral liver parts compared to controls.

On the cellular level the following was noticed in histological liver sections of 7, 10 and 14 days MSG treated embryos; the plasma membrane in most cells was not clear. Moreover many small clear vesicles appeared within the cellular cytoplasm, which gave the cytoplasm the granular appearance. Also an increase in the number and size of lipid droplets was seen in the treated sections compared to the controls. Changes were seen in the nucleus of hepatocytes of treated embryos. These were shrinkage in size, increased staining pyknotic (PY), karyolisis (KR). Also phagocytic cells were seen in blood sinusoids that had detached lining epithelial cells. Necrosis was also seen in treated sections. (Figs. 3-29,30,31 B).

4. Discussion

Glutamic acid is present naturally in several kinds of natural foods **(Garattini, 2000).** It is used by different body tissues such as muscles and liver (Munro 1979). Glutamic acid is considered to be a major excitatory transmitter within the brain **(Siegel, *et al.*, 1999)**. High levels of glutamic acid might cause hyper excitation and death of neurons **(Garattini, 2000)**. It also caused brain lesions in some animals **(Olney 1980).**

Many studies mentioned the adverse effects of MSG. Neonate rat liver (Battacharyo *et al*., 2011).

Nervous tissue in broiler chicks was affected when MSG was added to their food (Ati *et al*., 2000). Farr *et al*., 2010 suggested a relation between MSG and the nonalcoholic fatty liver syndrome in humans.

This study showed that MSG treated chick embryos had symptoms of growth retardations such as reduced whole body weight and length, neck length and beak length. The study also showed different congenital malformations in treated chick embryos such as abdominal hernia, bleeding, monophthalmia, and brain deformation. As much bleeding was seen in embryos when opening the eggs it could be concluded that inadequate amounts of blood reached the embryo therefore the amount of nutrients transferred from yolk to embryo was not enough, leading to growth retardation.

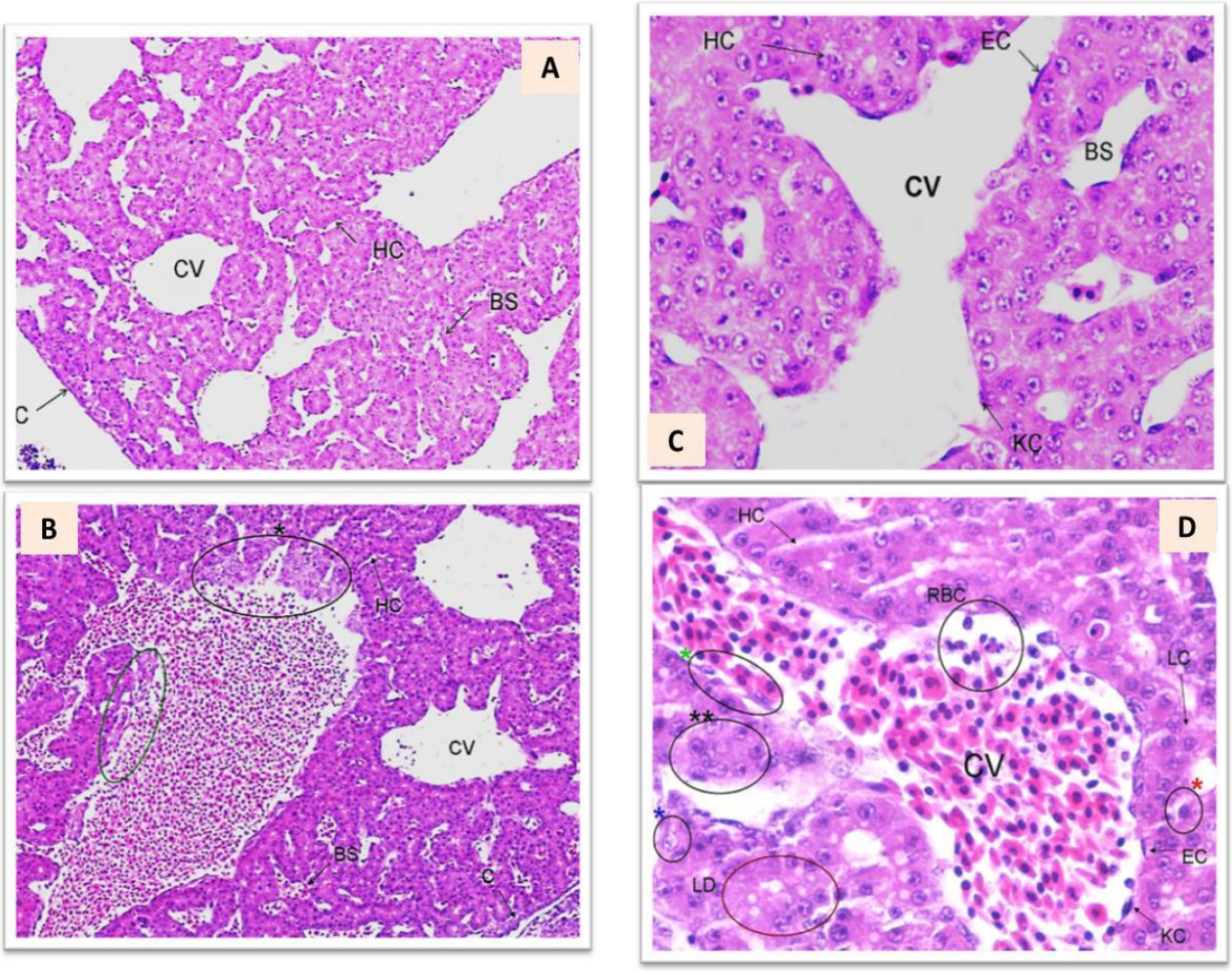

Figure 6 Showing 7 day chick embryo liver micrographs. (A and C) control, (B and D treated). (A&B 100X) (C&D 400X). (A&B&C&D HE)

(A) Shows that the control liver is surrounded by a connective tissue capsule. Hepatic cords (HC) with blood sinusoids in between them are arranged radially around the central vein (CV). (B) Liver is also surrounded by a connective tissue capsule .Note here that the hepatic cords are not very well distinguished compared to the controls. Also note the bleeding and congestion seen in the central vein. Fibrosis is seen in the inner layer of the central vein (green circle) also note the damage seen in the hepatocytes surrounding the central vein (*). (C) Very clear hepatic cords surrounding radially the central vein, blood sinusoids lined with endothelial cells and kupfer cells are also seen clearly. (D) Heavy bleeding is seen in the central vein filled with red blood cells. The plasma membrane is not clear in most hepatocytes (**) with Clear vacuoles seen in their cytoplasm. Lipid droplets have increased in number and volume compared to the controls. Karyolisis and pyknotic are seen in hepatocytes. Note the detached lining endothelial cells (green star)

Key: connective tissue capsule (C), central vein (CV), hepatic cords (HC), blood sinusoids (BS), lipid droplets (LD), kupfer cells (KC), endothelial cells (EC), red blood cells (RBCs).

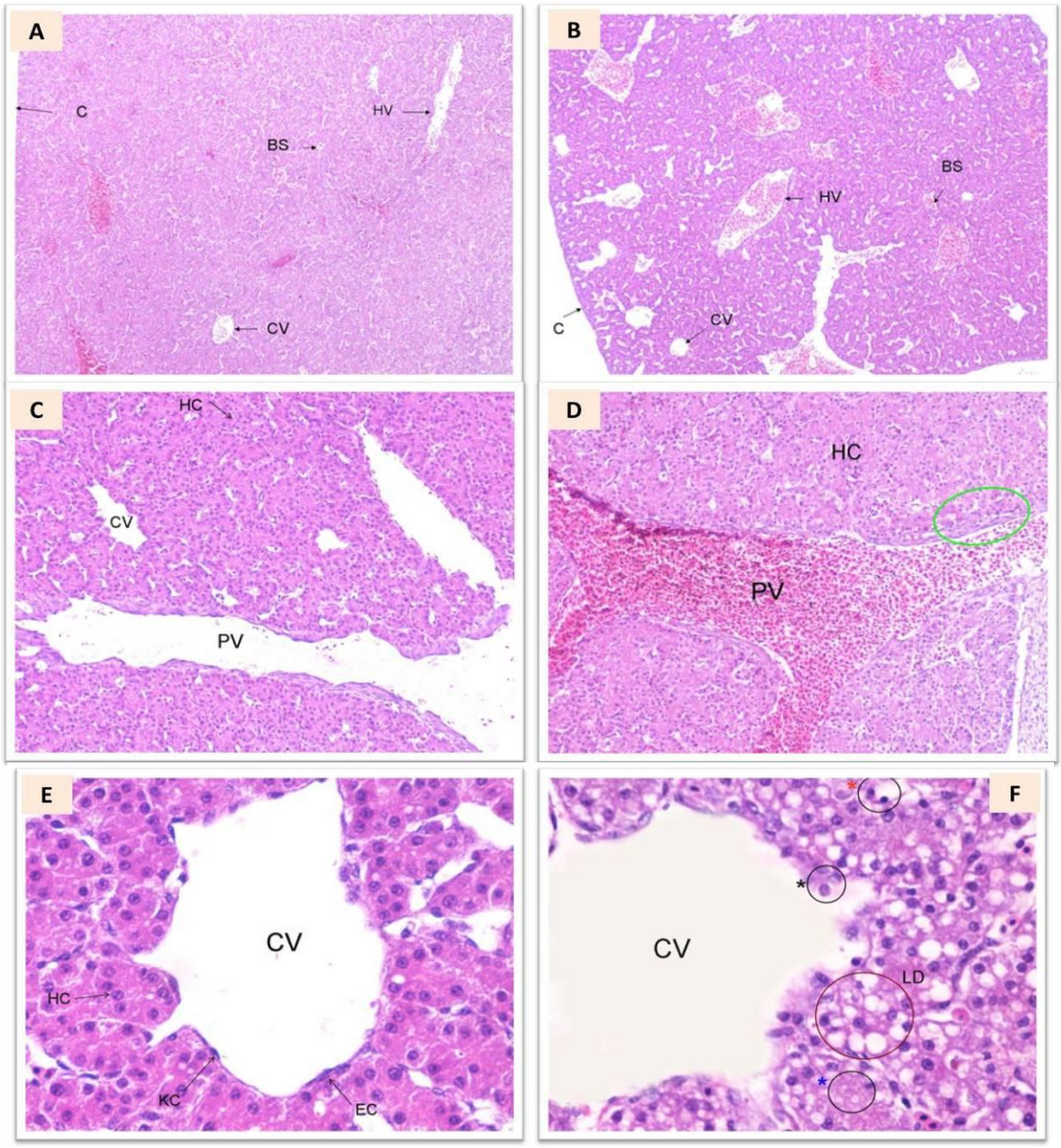

Figure 7 Showing 10 day chick embryo liver micrographs. (A, C and E) control, (B, D and F) treated. (A&B 40X), (C&D 100X), (E&F 400X) all are H&E stained. (A, C & E) The liver tissue seems to be denser than in control 7 day chick embryo liver. Blood sinusoids are seen, and hepatic cords are organized radially around the central vein. (B) Hepatic cords seem to be less dense compared to the control, bleeding could be seen in hepatic veins. (C) A portal vein could be seen. (D) Hepatic cords are distorted and not distinct compared to the control. The portal vein is very congested and filled with RBCs. Fibrosis (green circle) could be seen in the wall of the portal vein. (E) Endothelial cells and kupffer cells could be seen lining the central vein. (F) Distortion in liver tissue is seen. Pyknotic red star and karyolisis (blue star) could be seen within hepatocytes. Lipid droplets (red circle) are seen with an increase in number and volume compared to controls. Also some cell nucleus is seen protruding in the lumen of the central vein (black star)Key: connective tissue capsule (C), central vein (CV), hepatic cords (HC), blood sinusoids (BS), lipid droplets (LD), kupfer cells (KC), endothelial cells (EC), red blood cells (RBCs) portal vein (PV), hepatic vein (HV).

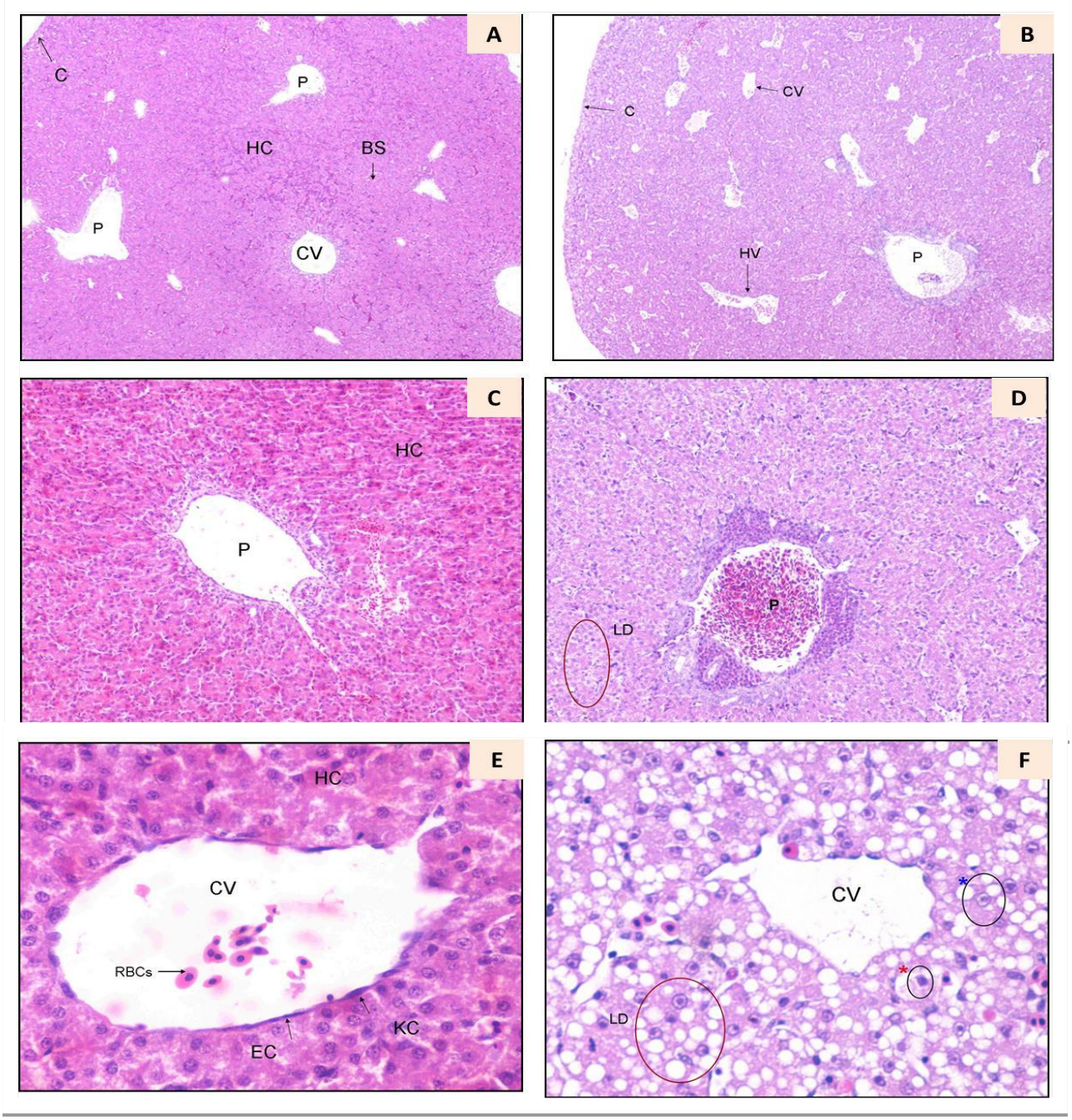

Figure 8 showing 14 day chick embryo liver micrographs. (A, C and E) control, (B, D and F) treated. (A&B 40X), (C&D 100X), (E&F 400X) all are H&E stained. (A &C) liver appears denser than previous ages (7 and 10 days) surrounded by connective tissue capsule. The hepatic cords and blood sinusoids are clear and are arranged radially around the central vein. The portal area is seen for the first time in this age. (B) Hepatic tissue appears less dense compared to the controls. Hepatic veins seem to be dilated and contain RBCs. The portal area seems distorted. (D) Portal area is congested and distorted with non-clear edges and filled with RBCs. Most hepatocytes surrounding the portal area seems to be damaged. Lipid droplets have increased in number and volume compared to the control. (E) The central vein appears lined with endothelial cels and kupffer cells and has some RBCs in its lumen. The hepatic cords are arranged radially around the central vein. (F) Hepatic cords are distorted. Hepatocytes deformed with no clear plasma membrane. Pyknotic (red star) and karyolisi (blue star) are seen within the hepatocytes. Also an increase in the number and volume of lipid droplets compared to the controls is clearly seen (red circle).

Many studies showed that MSG caused bleeding (Olney, 1969; Olney and Ho, 1970; Reynolds *et al*., 1976; Olney and Price, 1978; Krieger *et al*., 1979; Olney *et al*., 1980 ; Mosqueda-Garcia *et al*., 1986; Pilcher and Joseph, 1986; Park *et al*., 2000) Some studies showed that high levels of glutamate might block the central eye artery causing monophathalmia or anophthalmia (Wuu *et al*., 1988; Kawamura and Azuma, 1992; Sucher *et al*., 1997; Osborne *et al* ., 1999; vorwerk *et al* ., 1999; Tamas *et al* ., 2004).

This study showed that the control chick embryo liver consisted of two lobes, right and left connected with a middle piece. The right lobe appeared slightly bigger than the left lobe as seen from the body cross sections. Patten,1971 and Al-Gamdi, 2007 described the liver development of normal chick embryo . Their studies showed that during days 8 and 9 of incubation an expansion of hepatic cords was seen with less sinusoidal areas. In this study the same observation was seen in the liver sections of 10 day control chick embryo when compared to liver sections of 7 day control chick embryo. The current study showed that chick embryo liver consisted of hepatocytes arranged into hepatic cords which are arranged radially around the central veins, between hepatic cords, some blood sinusoids were also seen. Central veins and blood sinusoids were lined with endothelial cells (EC) and kupffer cells (KC). The same description was mentioned in other studies (Elnaggar, 1977; Abdel-fatah, 1992; Al-Gamdi 2007).

In this study lipid droplets were clearly seen in histological liver sections of 10 and 14 day control chick embryos. This was also mentioned in other studies (Abdel-fatah, 1992; Al-Gamdi 2007).

This study showed that injecting chick embryos once before incubation with 0.1 ml of the MSG solution (0.75mg MSG/gm. egg weight) caused hepatic tissue in all studied ages to be less condensed with more dilation in blood sinusoids and veins compared to the controls. These symptoms were also seen in other studies (Ortiz *et al*., 2006; Nakanishi *et al*., 2008). This study also showed steatosis in the liver tissue of treated chick embryos. The same observation was mentioned in the study of Nakanishi *et al*., 2008, where neonate males were treated with 2mg/gm body weight and this treatment was repeated for five days. This observation might indicate the severe effect of MSG on embryonic development as in this study the amount of MSG injected was very low (0.75mg MSG/gm. egg weight) injected once, compared to that of Nakanishi, *et al*., 2008 study (2mg/gm), yet a similar effect was seen in liver sections.

In this study an increase in the number of lipid droplets was seen in liver sections of treated 10 and 14 day chick embryos compared to the controls. Same symptoms were seen in other studies (Ortiz *et al*., 2006; Nakanishi *et al*., 2008). This study also showed liver cirrhosis and congestion of blood sinusoids and central vein in treated chick embryo liver tissue. Same observations were seen in the study of Ortiz *et al*., 2006.

The importance of this study raises from two factors the fact that the used dose is very small compared to other used doses, and administrating the dose while the embryonic development is taking place and studying the effect on different ages during embryonic development.

More studies should be made as to understand the mechanism undertaken by MSG to affect embryonic development in order to be able to know its precise effect on human embryo development if taken by pregnant women.

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