

Original Article	Effects Of Monosodium Glutamate On Hippocampus Development In Albino Rats And Pregnant Mothers <i>Asmaa Mohammed Tolba M. Abdel-Aal</i> <i>Anatomy and Embryology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt</i>
-------------------------	--

ABSTRACT

Monosodium glutamate (MSG) is a sodium salt derivative of a natural amino acid called glutamic. The use of monosodium glutamate is very controversial in recent years. The aim of this study was to investigate the MSG administration effects on the hippocampus development in the prenatal and postnatal periods as well as its neurotoxic effects on a pregnant mother hippocampus. Sixty pregnant albino rats were used in this study and were divided into 3 equal groups: Group I, control group, Group II, pregnant mothers treated with MSG in a dose of 830 mg/Kg body weight per day orally from gestation day 0 to 19th, they were sacrificed at gestation day 20 for prenatal histological examination of fetuses brains. Group III, pregnant animals were treated with oral MSG 830 mg/Kg body weight, from the gestation day 0 and they were allowed to deliver their neonates. The mothers were maintained at the same dose of MSG orally for three weeks after delivery and their neonates were kept on breast feeding up to the age of weaning. At weaning, all neonates (males and females) of the treated mothers were sacrificed and sections of brain were stained with hematoxylin and eosin (H&E) for examination. Immunohistochemical detection of Glial fibrillary acidic protein (GFAP) and caspase-3 were detected in the brain sections of the fetuses of the group (II), neonates of the group (III) and treated mothers for evaluation of the effects of MSG intake on the hippocampus of the fetuses, the neonates and the mothers. Examination of brain sections from fetuses of (group II) treated mothers with MSG showed an alterations in the brain development compared to the fetuses of the control group (group I). Examination of the brain sections from neonates of (Group III) treated mothers with MSG as compared to the neonates of the control group (group I) revealed gliosis formed by glial cell proliferation and degenerated nerve cells. Multicystic encephalomalacia and cellular neurodegenerative changes in the hippocampus of the pregnant mothers were also observed. The statistical analysis of area percent of the GFAP and the caspase-3 immunoexpression in the brain sections of the fetuses of the group (II) and the neonates of the group (III) MSG- treated animals showed a significant increase as compared with the control group (I). Also, MSG- treated mothers showed a significant increase of the area percent of GFAP and caspase-3 immunoexpression compared with the control mothers group. Maternal administration of MSG during pregnancy and lactation has potent neurotoxic effects on brain development of the fetuses and neonates. Therefore, MSG intake should be avoided in the pregnancy and lactating periods because the hippocampus of the developing neonates and fetuses were affected by MSG.

Key Words: Development, hippocampus, monosodium glutamate, rat

Corresponding Author: Asmaa Mohammed Tolba Mohammed Abdel-Aal, Anatomy and Embryology department, Faculty of medicine, Zagazig University, **Email:** amtolbaa@zu.edu.eg, **Mobile:** 01142203098

INTRODUCTION

The Monosodium glutamate (MSG) is one of the popular flavor enhancer used by various food industries (Veni *et al.*, 2010). Use of MSG as a flavor has been questioned due to its toxic effects, but in many countries, there are no limitations on the amount of MSG that can be added to food (Sant'Diniz *et al.*, 2005). The average human daily intake of MSG in industrialized countries is 0.3 to 1 gm and in a highly seasoned restaurant meal as much as 5 gm may be ingested. MSG

produce human symptom complex such as numbness, weakness, flushing, sweating, dizziness, and headache in an apparent threshold dose of 2.5 and 5 gm MSG (Yang *et al.*, 1997). The central nervous system is an important target organ for the action of MSG, particularly during brain development. The mechanisms by which MSG exerts its actions on the nervous system are not yet fully clarified (Hermanussen and Tresguerres, 2005). Excessive amounts of glutamate excitatory neurotransmitter may act as a potent neurotoxin through increasing

the excitability and activating the proteolytic enzymes (Weil *et al.*, 2008). Increasing brain glutamate levels during development has affected neuronal differentiation and circuitry formation (Lopez-Perez *et al.*, 2010). MSG can affect the morphological and electrophysiological organization of the brain, and this effect is more severe during brain development (Lima *et al.*, 2013). Rats subjected to monosodium glutamate administration during the neonatal period display a chronic impairment of the hippocampus synaptic plasticity (Sanabria *et al.*, 2002). MSG cause damage to areas of the brain unprotected by the blood brain-barrier (Gudiño-Cabrera *et al.*, 2014). Both the young and the elderly are at risk from MSG as the blood-brain barrier is not fully developed in the young and damaged by aging in the elderly (Meldrum, 1993).

Walker and Lupien, (2000) reported that the blood-brain barrier may be less effective in the neonatal mouse, however, the question of the comparability of the rat and the human infant remains an issue, as the level of brain development in the two species is quite different. The hippocampus is a region of the mammalian brain arises from the medial region of the subventricular zone within the telencephalon in which new neurons are continually generated in the postnatal period and throughout life (Yamada *et al.*, 2015). The hippocampus participates in biological functions including motivation, spatial navigation, adaptive timing and declarative memory (Tracy *et al.*, 2001). The astrocytes are the most abundant specialized glial cell type in the central nervous system which is required for the proper complex physiological functions and survival of neurons (Sofroniew and Vinters, 2010). The glial fibrillary acidic protein (GFAP) is the principal intermediate filament protein of mature astrocytes and is considered to be relatively astrocyte - specific marker involved in controlling the shape and movement of astrocytes and plays a vital role in modulating synaptic efficacy in the central nervous system (Higashino *et al.*, 2009). GFAP is not present throughout astrocyte cytoplasm but only in the main branches (Sofroniew and Vinters, 2010). The expression of GFAP is essential for the process of reactive astrogliosis and glial scar formation (Herrmann *et al.*, 2008). These astrocyte scars in severe tissue damage act as protective barriers to inflammatory cells and infectious agents (Sofroniew and Vinters,

2010). Decreased GFAP expression compromise neuronal survival and were associated with detrimental conditions in the central nervous system (Giffard and Swanson, 2005).

AIM OF WORK

This study aimed to investigate the possible toxic effects of MSG, as a natural constituent of many food items, on the hippocampus development of albino rats and their mothers during pregnancy. This was done by the histological study using (H&E) stain and immunohistochemical detection of the GFAP and the caspase-3.

MATERIAL AND METHODS

Eighty adult albino rats, twenty males and sixty females with an average weight of 200-220 grams each, were used in this study. They were obtained from the Laboratory Animal Unit, Faculty of Medicine, Zagazig University. They were kept in fan ventilated wide polypropylene animal cages, under the prevailing environmental conditions at the room temperature under pathogen-free conditions. Their food was a balanced diet in the form of barely, lettuce, milk, carrots, bread and water. Before the experiment, rats were acclimatized to the experimental conditions for a period of one week. All rats were handled accordance to the standard guide for the care and use of laboratory animals. Adult females were housed with adult males at a ratio of 2: 1 respectively at night in each cage for mating. The vaginal smears were taken in next day from the adult females to detect the occurrence of pregnancy. If the vaginal smear in the following morning contained sperms, that day is considered as the day zero of gestation. After coupling, sixty pregnant rats were obtained and were used as the experimental animals in this study. Pregnant female rats were being equally divided into three groups as follows: twenty of them were isolated as the control group (group I) and forty pregnant rats (n=40) were divided into 2 equal groups (group II & III). Group II & III were treated with MSG (C₅H₉NO₄.Na) obtained from (El Nasr Pharm. Chem. Co., Egypt) in a dose of 830 mg/kg body weight (Abass and El-Haleem, 2011). The selected dose was 1/20th of LD₅₀. The dose was selected after finding out the acute oral LD₅₀ value in rats which was found to be 15.000-18.000 mg/kg body weight (Walker and Lupien, 2000).

Group I: (Control group)

Twenty of the pregnant mothers (n=20) were separated as a control group which had received an ordinary food without any additives and normal saline orally via intragastric tube daily. Half of the animals (n=10) were sacrificed at gestation day 20 for prenatal brain examination of the fetuses. Also, brain specimens were collected from the group (I) pregnant females to examine the hippocampus of the pregnant mothers. The other half of the animals were allowed to complete their term and deliver their neonates which were maintained on breastfeeding up to the age of weaning 3 weeks. At weaning, all neonates (males and females) were sacrificed for postnatal brain examination.

Group II: (Prenatal treated group)

This group contained twenty pregnant mothers (n=20) which were orally exposed to (830 mg/kg body weight) MSG from the gestation day 0 to 19th. The animals of the group (II) were sacrificed at gestation day 20 for prenatal brain examination of fetuses. Also, brain specimens collected from the group (II) pregnant females to examine the MSG effects on pregnant mothers hippocampus.

Group III: (Postnatal treated group)

This group contained twenty pregnant mothers (n=20) which were treated with MSG (830 mg/kg) orally from the gestation day 0 until D21 after birth. The treated mothers were allowed to deliver naturally and each mother was housed with the neonates in a large cage in a ventilated room at a constant temperature (25°C) with a 12:12 h light/dark cycle. The mothers were maintained at the same dose (830 mg/kg) of MSG orally for 3 weeks during lactation and their neonates were kept on breastfeeding up to the age of weaning. Monosodium glutamate passed to the newborns through their mother's milk. At weaning, all neonates of the treated mothers (n=30) of both sexes were sacrificed for evaluation the effect of MSG intake during pregnancy and lactation on hippocampus development of the neonates. Brains were taken out, bisected along the midline, and fixed in 10% buffered formalin 6 hours. Specimens from the brains were processed to prepare 5 µm paraffin sections stained with hematoxylin and eosin stain for the Light microscopic examination (Bancroft and Gamble, 2002).

GFAP and caspase-3 immunohistochemical detection

The immunohistochemical glial fibrillary acidic protein (GFAP) and caspase-3 localization using avidin–biotin–complex (ABC) immunoperoxidase technique. The sections were incubated in hydrogen peroxide for 10 min to block the endogenous peroxidase then incubated with solution of primary antibodies 20 min at room temperature (the primary anti-GFAP antibody at 1:100 dilutions; Caspase-3 antibodies 3 µg/ml with dilution 1: 200). For the primary antibody used was a mouse monoclonal antibody (Glial Fibrillary Acidic Protein) Ab-1 (Clone GA-5), specific to the astrocytes obtained from Lab Vision Corporation, Medico Co., Egypt (Cat. #MS-280-B0). The Caspase-3 antibody react broadly with all known caspase-3 variants of human, rat and mouse origin by immunohistochemistry (Lab Vision Corporation, USA). Then the slides were washed with diluted phosphate buffered saline (PBS) then incubated with the secondary anti-mouse antibodies universal kits for 30 minutes in a humid chamber at room temperature. Staining was completed by incubation with substrate chromogen 3, 3'Diaminobenzidine (DAB) for 5–10 min. One DAB tablet was dissolved in 10 ml PBS. In a separate tube, 0.2ml hydrogen peroxide was added to 5.8 ml distilled water. Then, 0.2 ml diluted hydrogen peroxide solution was added to DAB solution and mixed well. Chromogen resulted in brown-colored precipitate at the antigen sites and Mayer's Haematoxylin was used as a counter stain. Positive control was IMR5 cells in the brain. For negative controls, incubation was carried out with the omission of the primary antiserum. The positive reactivity of GFAP and caspase-3 staining were exhibited as different grades of reactivity (i.e. weak, moderate and strong) according to the intensity of staining. The positive reactivity of GFAP and caspase-3 were indicated by brown color reaction (Kiernan, 2000).

GFAP and caspase-3 stained sections; quantitative measurements:

Immunohistochemical GFAP and Caspase-3 stained sections were morphometrically analyzed using image analyzer computer system. The quantitative morphometric measurements were achieved by using the (Leica Qwin standard

software, colored monitor, digital camera CH-9435 DFC 290 coupled to the microscope, made in Germany, in the Pathology department, Faculty of Dentistry, Cairo University). The image analyzer was first calibrated automatically to perform the measurement in units (pixels). This image analyzer was used to measure the area percent of immunological reaction for the GFAP and the Caspase-3 in the dentate gyrus region. The standard measuring frame of an area equal to 118476.6 μm^2 was chosen from the parameters using measuring field menu. These measurements were done using an objective lens of total magnification 400 exhibiting positive reactivity with the accumulation of all grades of reactivity (i.e. weak, moderate and strong), (Bocci *et al.*, 2001). The data obtained, the area percent of the GFAP reaction and the caspase-3 were subjected to statistical analysis using SPSS statistical software (SPSS for Windows, version 13.0).

In each specimen, the readings were obtained and the mean values and standard deviations (SD) were calculated automatically. Comparison between the control group (I) and the fetuses of group (II) and the neonates of group (III) MSG treated mothers was done. Also, the adult mothers treated with MSG (group II) compared with the control mothers were made using the t-test. Data were expressed as mean (\pm) SD. Results were considered significant when P value is < 0.05 .

RESULTS

I- light microscopic examination

Light microscopic examination of brain sections from control fetuses (group I) at gestation day 20 showed normal immature granular neurons around the ventricular indentation with no pathological changes (Fig. 1). Examination of brain sections from fetuses of (Group II) treated with MSG compared to the control group showed degenerated neuroepithelial cells with pyknotic nuclei surrounded by spaces forming lacunae and astrocytic hyperplasia (astrocytosis) that was characterized by increased number of the proliferative astrocytes (Fig. 2). Gemistocytes had developed within the brain tissue of the fetuses in the region of the developing hippocampus. The gemistocyte was a swollen reactive astrocyte with a large cytoplasmic mass and increased cytoplasmic filaments. Such astrocytes were also known as gemistocytes.

Also, eosinophilic granular bodies (Rosenthal fibers) were observed together with these gemistocytes. The Rosenthal fiber was a thick elongated corkscrew eosinophilic (pink) bundle that was found in the presence of reactive gliosis (Fig. 3). The multicystic encephalomalacia was noticed and was characterized by glial cell proliferation (gliosis) clumped around the damaged hippocampal tissue leading to variable size cyst formation which were communicating with each other (Fig. 4). Light microscopic examination of brain sections of control neonates of (group I) animals showed the hippocampus had 3 areas: CA1, CA2, CA3 and the dentate gyrus. CA1, CA2 and CA3 of the hippocampus composed of four layers: Oriens, pyramidal cell layer, Stratum radiatum and the lacunosum moleculare layer. The oriens and the molecular layers were formed of few cells, whereas the pyramidal layer was formed of numerous rounded nerve cells with large vesicular nuclei and pale basophilic cytoplasm (Fig.). The dentate gyrus was the region which projected into the floor of the lateral ventricle. The dentate gyrus composed of three layers: granular, molecular and polymorphic (Fig.). Brain sections from neonates of (Group III) animals treated with MSG revealed spaces forming lacunae in the CA1 and CA2 around the nerve cells in the oriens, molecular and the pyramidal layers (Fig. 7). Gliosis formed by glial cell proliferation in the granular layer of the dentate gyrus (Fig. 8). Encephalomalacia was observed in the pyramidal layers of the CA2 and CA3 regions (Fig. 9). Examination of brain sections of the control neonates of group (I) showed normal Virchow–Robin space which is a perivascular space surrounded intact blood vessels (Fig. 10). However, the brain sections of the neonates of (group III) MSG-treated animals revealed hemorrhage in the Virchow–Robin space (Fig. 11). Light microscopic examination of sections from the hippocampus of the control adult mothers group (I) was C-shaped projected into the floor of the temporal horn of the lateral ventricle which was formed from CA1, CA2, CA3 and the dentate gyrus. Each part of the CA1 and CA2 was composed of four layers: oriens, pyramidal cell layer, Stratum radiatum and lacunosum moleculare layer. The dentate gyrus consisted of three layers: granular, molecular and polymorphic (Fig. 12). The molecular and granular layers of the dentate gyrus were formed from regular closely packed intact

nerve cells (Fig. 13). The pyramidal layer in the CA1 and CA2 was formed of numerous regular arranged closely packed rounded nerve cells with large vesicular nuclei and pale basophilic cytoplasm (Figs. 14 & 15). The pyramidal layer in the CA3 of control adult mother hippocampus was regular closely packed intact nerve cells (Fig.12). The astrocytes presented in between the pyramidal cells in the regular pyramidal cell layer in the CA1 region of control adult mother hippocampus (Fig.15). The hippocampus in the adult mothers treated with MSG (group II) showed irregular arrangement of degenerated dispersed nerve cells with pyknotic nuclei in the pyramidal layer of the CA1 and CA2 regions and were surrounded by lacunar spaces in the oriens, the stratum radiatum, the molecular and the pyramidal cell layers (Fig.16). Multicystic encephalomalacia was characterized by glial cell proliferation around the damaged hippocampal tissue in the oriens, the stratum radiatum, the molecular and the pyramidal cell layers of the CA1, CA2 (Figs. 16&17) and CA3 region (Fig.19), leading to a variable size wide cyst formation communicating with each other. The cystic encephalomalacia in the granular and the molecular layers of the dentate gyrus was characterized by an irregularly disturbed arrangement of the granular cell layer of the dentate gyrus and appearance of wide cystic spaces in between the granular cells which extended into the molecular and polymorphic layers of the dentate gyrus and were surrounded by glial cell proliferation (Figs. 18 & 19).

II-Immunohistochemical results of GFAP reactivity

Examination of brain sections of control fetuses (group I) at gestation day showed mild positive GFAP reactivity at this prenatal age (Fig. 20). Examination of brain sections from fetuses of (Group II) treated with MSG showed a dense positive astrocytic reaction in the hippocampus as compared to the control group (group I), (Fig.21). Brain sections from the control neonate group showed mild positive GFAP immunoreaction localized in the short processes of astrocytes (Fig.22). Brain sections from the neonates of Group III (treated animals

with MSG) showed widely distributed intense GFAP immunoreactive astrocytes in CA1 & CA2 and CA3 and diffuse reactive astrogliosis developed in the region of the dentate gyrus that was characterized by an increased glial fibrillary acidic protein and astrocytes proliferation and their processes were long and interwoven with each other in the molecular and polymorphic layers and extended into the granular layer leading to scar formation (Fig.23). Examination of brain sections from the adult mother hippocampus control group showed a mild positive GFAP immunoreaction localized in the short processes of the astrocytes in the dentate gyrus (Fig. 24). Examination of brain sections from the adult mothers treated with MSG (group II) revealed intense GFAP immunoreactive astrocytes in the dentate gyrus of the hippocampus (Fig. 25).

III-Immunohistochemical results of Caspase-3 reactivity

The immunohistochemical detection of Caspase-3 showed a strong positive immunoreactive staining among the MSG-treated groups. Examination of brain sections of control fetuses (group I) at gestation day 20 showed slightly faint reactivity at this prenatal age (Fig.26). Examination of brain sections from fetuses of (Group II) showed a strong positive Caspase-3 reaction in the region of the hippocampus (Fig.27). Brain sections from the neonates of control group (group I) showed a slightly faint reactivity in the dentate gyrus region of the hippocampus (Fig.28). Brain sections from the neonates of (Group III) showed a widely distributed strong Caspase-3 immunoreactivity in the granular and polymorphic layers of the dentate gyrus region of the hippocampus (Fig.29). Examination of brain sections from the adult mother hippocampus control group showed negative Caspase-3 immunoreaction localized in the dentate gyrus of the hippocampus (Fig.30). However, examination of brain sections from the adult mothers treated with MSG (group II) revealed strong immunoreactions of the Caspase-3 in the dentate gyrus region in the hippocampus (Fig.31).

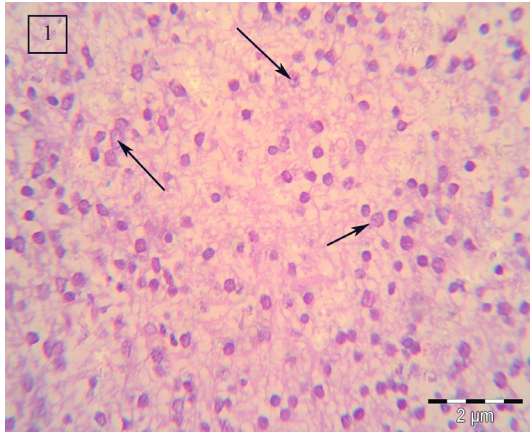


Fig. 1: A photomicrograph of a section in the hippocampus of control fetuses at gestation day 20 showing normal granular neurons (arrows). (Hx. & E.; X 400 .Scale bar = 2 μm).

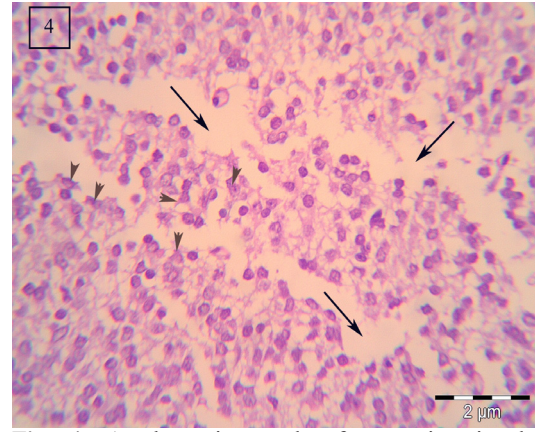


Fig. 4: A photomicrograph of a section in the hippocampus of fetuses of (Group II) treated with MSG showing multicystic encephalomalacia (arrows) with gliosis (arrow heads). (Hx. & E.; X 400 .Scale bar = 2 μm).

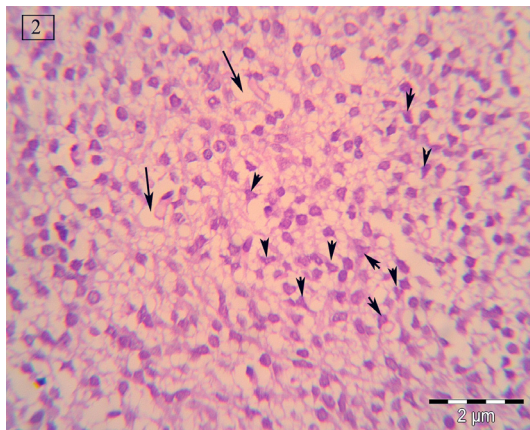


Fig. 2: A photomicrograph of a section in the hippocampus of fetuses of (Group II) treated with MSG showing degenerated neuroepithelial cells surrounded by spaces forming lacunae (arrows) and astrocytic hyperplasia (arrow heads). (Hx. & E.; X 400 .Scale bar = 2 μm).

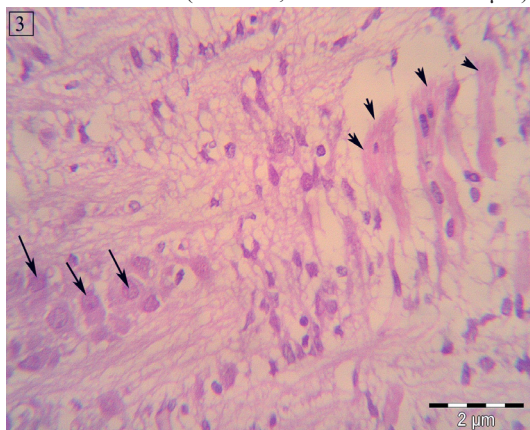


Fig. 3: A photomicrograph of a section in the hippocampus of fetuses of (Group II) treated with MSG showing gemistocytes (arrows) and eosinophilic granular Rosenthal fibers (arrow heads). (Hx. & E.; X 400 .Scale bar = 2 μm).

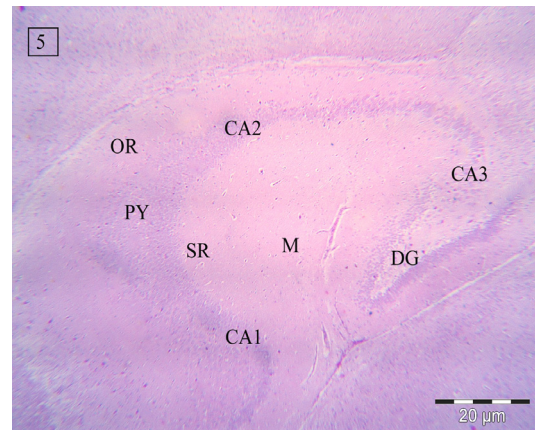


Fig. 5: A photomicrograph of a section in the hippocampus of control neonates showing CA1, CA2, CA3 and the Dentate gyrus (DG). CA1 composed of four layers: oriens (OR), pyramidal cell layer (PY), stratum radiatum (SR) and molecular layer (LMol). (Hx. & E.; X 40 .Scale bar = 20 μm).



Fig. 6: A photomicrograph of a section in the hippocampus of control neonates showing the dentate gyrus projected into the lateral ventricle (LV) composed of three layers: the granular (G), the molecular (M) and the polymorphic (P). (Hx. & E.; X 100 .Scale bar = 10 μm).

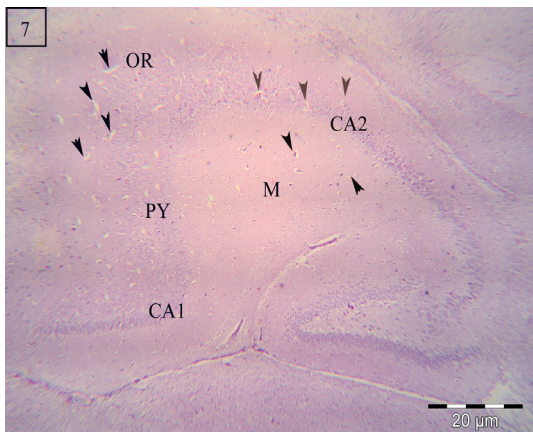


Fig. 7: A photomicrograph of a section in the hippocampus of neonates of (group III) treated with MSG showing lacunae (arrows) around the nerve cells in the pyramidal (PY), oriens (OR) and molecular (LMol) layers in the CA1 and CA2. (Hx. & E.; X 40. Scale bar = 20 μm).

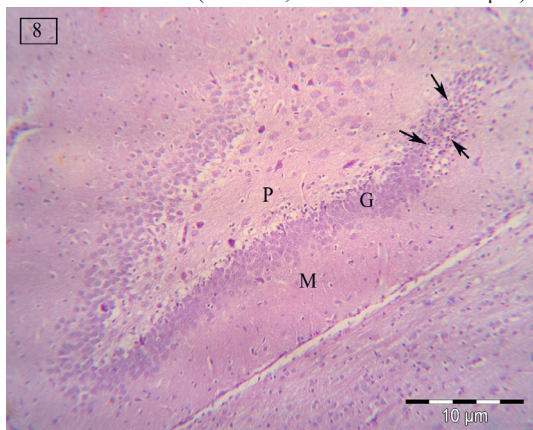


Fig. 8: A photomicrograph of a section in the hippocampus of neonates of (group III) treated with MSG showing gliosis (arrows) in the granular layer (G) between the molecular (M) and the polymorphic (P) layer of the dentate gyrus. (Hx. & E.; X 100. Scale bar = 10 μm).

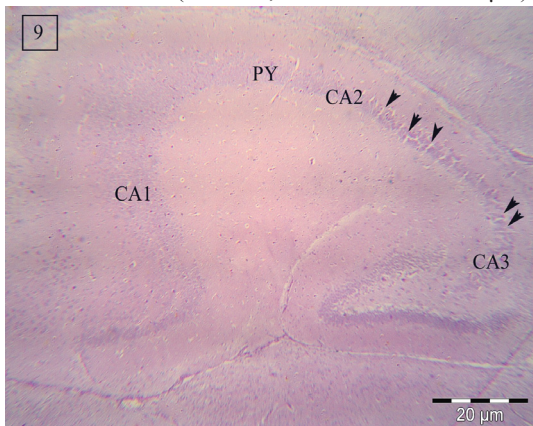


Fig. 9: A photomicrograph of a section in the hippocampus of neonates of (group III) treated with MSG showing CA1, CA2 and CA3. Encephalomalacia (arrows) was observed in the pyramidal layer (PY) of the CA2 and CA3. (Hx. & E.; X 40. Scale bar = 20 μm).

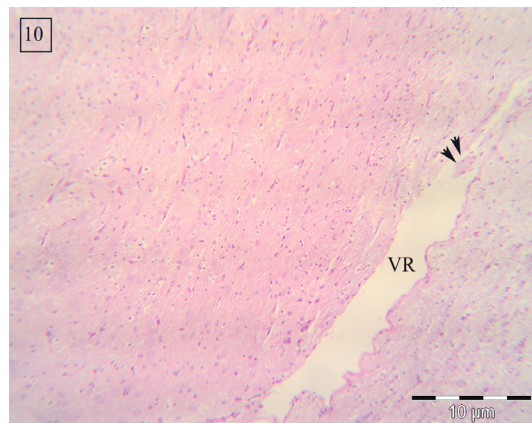


Fig. 10: A photomicrograph of a section in the hippocampus of control neonates showing the Virchow-Robin space (VR) which is a normal space surrounded the intact blood vessel (arrows). (Hx. & E.; X 100. Scale bar = 10 μm).

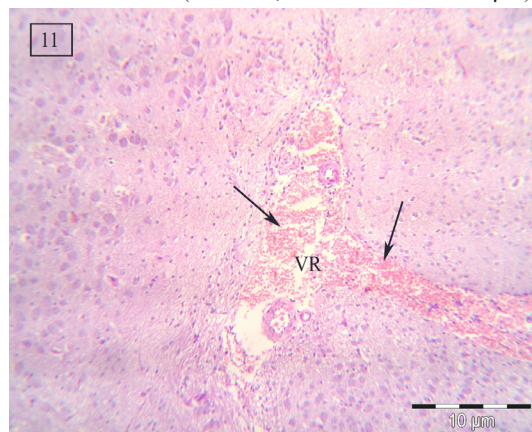


Fig. 11: A photomicrograph of a section in the hippocampus of neonates of (group III) treated with MSG showing hemorrhage (arrows) in the Virchow-Robin space (VR). (Hx. & E.; X 100. Scale bar = 10 μm).

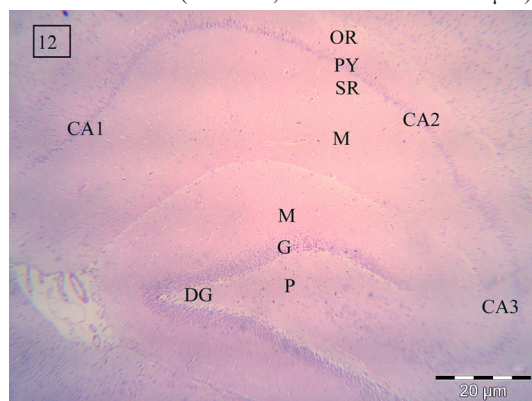


Fig. 12: A photomicrograph of a section in the hippocampus of control mothers showed CA1, CA2, CA3 and the dentate gyrus (DG). CA1 and CA2 composed of four layers: Oriens (OR), pyramidal cell layer (PY), Stratum radiatum (SR) and molecular layer (LMol). The dentate gyrus formed from three layers: granular (G), molecular (M) and polymorphic (P). (Hx. & E.; X 40. Scale bar = 20 μm).

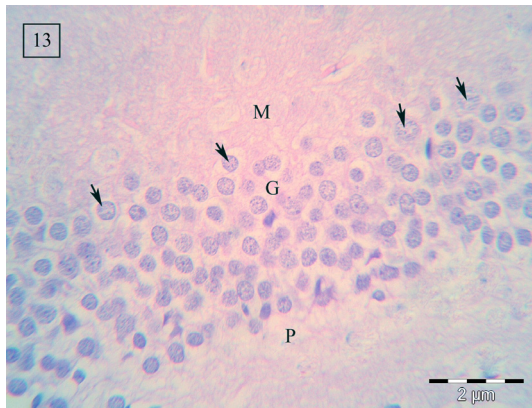


Fig. 13: A photomicrograph of a section in the hippocampus of control mothers showing granular layer (G) of the DG are formed from regular closely packed intact cells (arrows) between the molecular (M) and the polymorphic layer (P). (Hx. & E.; X 400 .Scale bar = 2 μm).

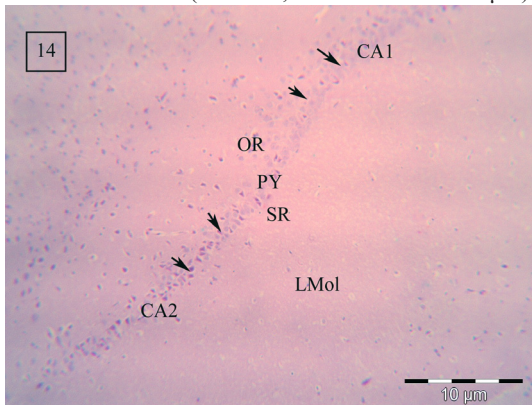


Fig. 14: A photomicrograph of a section in the hippocampus of control mother showing the molecular layer (LMol) and pyramidal layer (PY) in the CA1 and CA2 regions, the pyramidal layer was formed of regularly arranged closely packed rounded nerve cells (arrows) with large vesicular nuclei and pale basophilic cytoplasm between the oriens (OR) and the stratum radiatum (SR). (Hx. & E.; X 100. Scale bar = 10 μm).

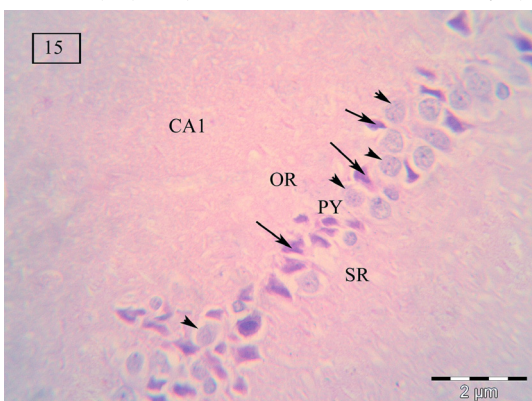


Fig. 15: A photomicrograph of a section in the hippocampus of control mothers showing the astrocytes (arrows) presented in between the pyramidal cells (arrow heads) in the regular pyramidal cell layer (PY) between the oriens (OR) and the stratum radiatum (SR) in the CA1 region. (Hx. & E.; X 400 .Scale bar = 2 μm).

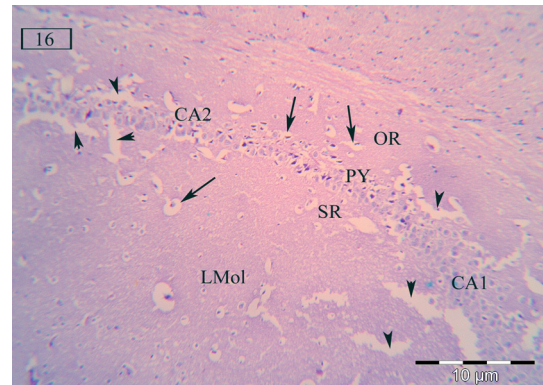


Fig. 16: A photomicrograph of a section in the hippocampus of MSG treated mothers showing cystic encephalomalacia (arrow heads) and degenerated nerve cells surrounded by lacunar spaces (arrows) in the oriens (OR), the pyramidal cell layer (PY), the stratum radiatum (SR) and the molecular layers (LMol) of the CA1 and the CA2. (Hx. & E.; X 100 .Scale bar = 10 μm).

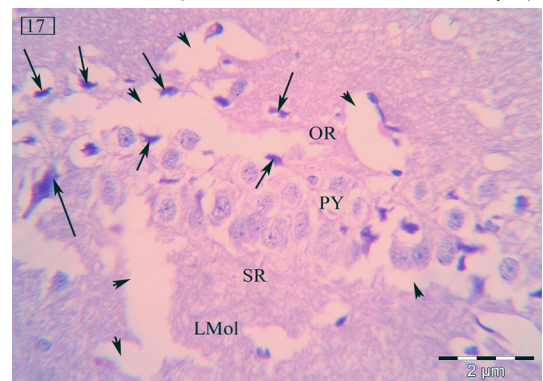


Fig. 17: A photomicrograph of a section in the hippocampus of MSG treated mothers showing irregular disturbed arrangement of degenerated dispersed pyramidal nerve cells with pyknotic nuclei in the pyramidal cell layer (PY) and cystic encephalomalacia (arrow heads) surrounded by astrocytes (arrows) around the damaged hippocampal tissue in the oriens (OR), stratum radiatum (SR) and the molecular (LMol) layers. (Hx. & E.; X 400 .Scale bar = 2 μm).

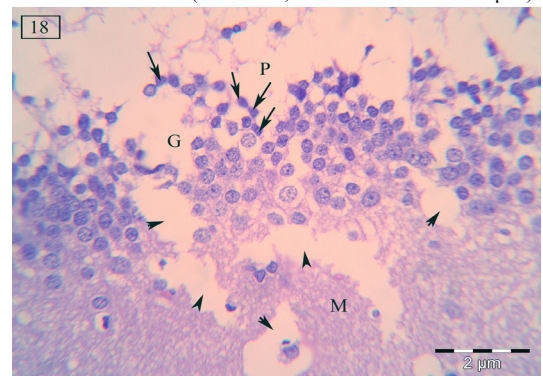


Fig. 18: A photomicrograph of a section in the hippocampus of MSG treated mothers showing cystic encephalomalacia (arrow heads) surrounded by glial cells (arrows) in the irregular disturbed granular (G), molecular (M) and polymorphic (P) layers of the dentate gyrus. (Hx. & E.; X 400 .Scale bar = 2 μm).

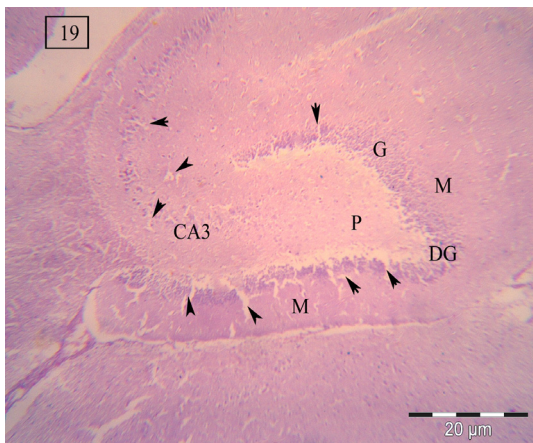


Fig. 19: A photomicrograph of a section in the hippocampus of MSG treated mothers showing cystic encephalomalacia (arrow heads) in CA3 region and their irregular disturbed granular (G), molecular (M) and polymorphic (P) layers of the dentate gyrus (DG). (Hx. & E.; X 40. Scale bar = 20 µm).

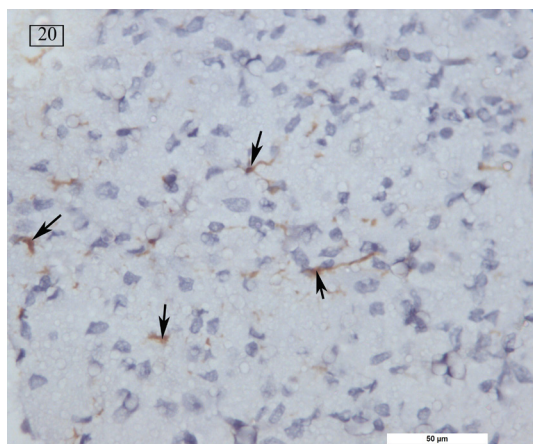


Fig. 20: A photomicrograph of a section in the hippocampus of control fetuses (group I) at gestation day 20 showing mild positive expression of the Glial Fibrillary Acidic Protein (GFAP) (arrows). (Hx. & E.; X 400. Scale bar = 50 µm).

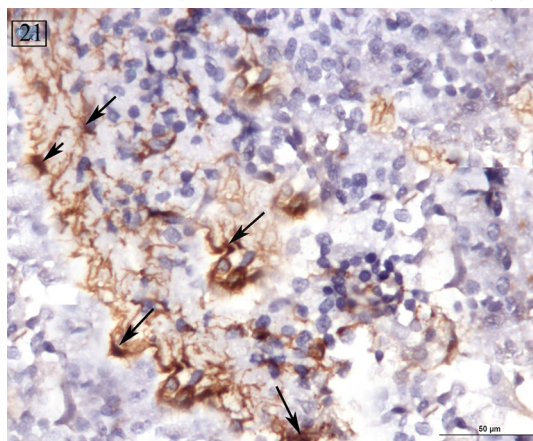


Fig. 21: A photomicrograph of a section in the hippocampus of the fetuses of (group II) treated with MSG showed a dense positive astrocytic reaction (arrows). (Hx. & E.; X 400. Scale bar = 50 µm).

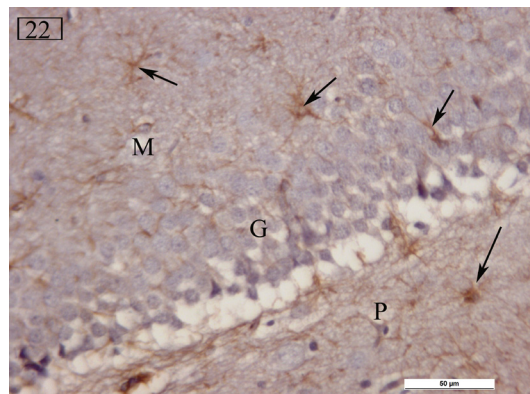


Fig. 22: A photomicrograph of a section in the hippocampus of the neonates of control group (group I) showed mild positive GFAP immunoreactions (arrows) localized in the short processes and bodies of the astrocytes in the molecular (M), polymorphic (P) and the granular layers (G) of the dentate gyrus. (Hx. & E.; X 400. Scale bar = 50 µm).

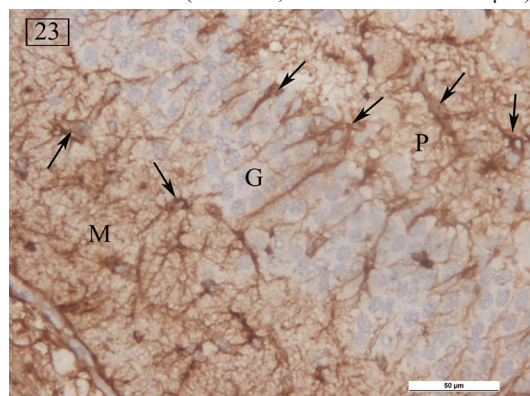


Fig. 23: A photomicrograph of a section in the hippocampus of the neonates of (group III) treated with MSG showed widely distributed intense GFAP reactive astrocytes and diffuse reactive astrogliosis (arrows) developed in the molecular (M), polymorphic (P) and the granular layer (G) in the region of the dentate gyrus led to scar formation. (Hx. & E.; X 400. Scale bar = 50 µm).

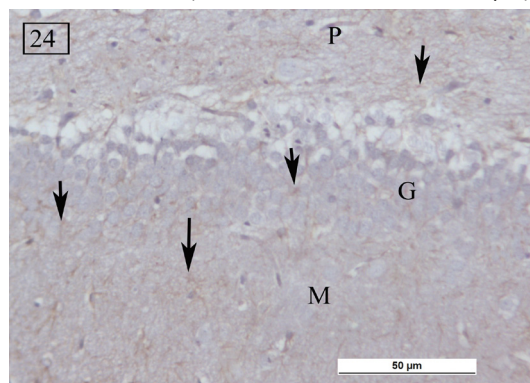


Fig. 24: A photomicrograph of a section in the hippocampus of the adult mothers control group showed mild positive GFAP immunoreaction (arrows) localized in the short processes of the astrocytes in the molecular (M), polymorphic (P) and the granular layers (G) of the dentate gyrus region. (Hx. & E.; X 400. Scale bar = 50 µm).

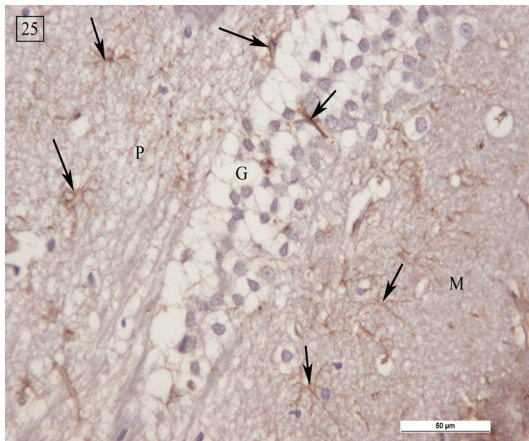


Fig. 25: A photomicrograph of a section in the hippocampus of the adult mothers treated with MSG (group II) revealed intense GFAP immunoreaction in the processes and the bodies of the astrocytes (arrows) in the molecular (M), polymorphic (P) and the granular layers (G) of the dentate gyrus region. (Hx. & E.; X 13 400. Scale bar = 50 μm).

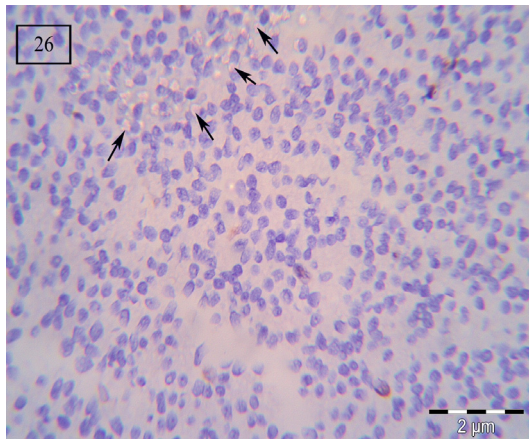


Fig. 26: A photomicrograph of a section in the hippocampus of control fetuses showing slightly faint reactivity of Caspase-3 staining (arrows). (Hx. & E.; X 400. Scale bar = 2 μm).

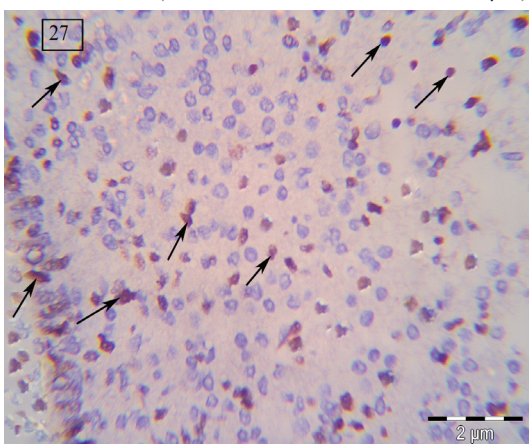


Fig. 27: A photomicrograph of a section in the hippocampus of fetuses of group (II) treated with MSG showing a strong positive caspase-3 staining (arrows). (Hx. & E.; X 19 400. Scale bar = 2 μm).

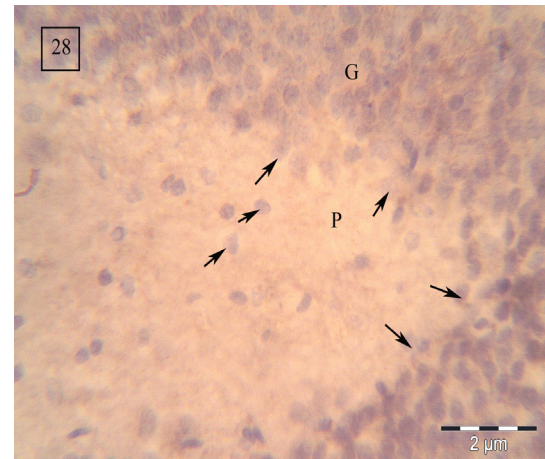


Fig. 28: A photomicrograph of a section in the hippocampus of control neonates showing negative caspase-3 immunoreactive cells (arrows) in the granular layer (G) and polymorphic layer (P) of the dentate gyrus region. (Hx. & E.; X 400. Scale bar = 2 μm).

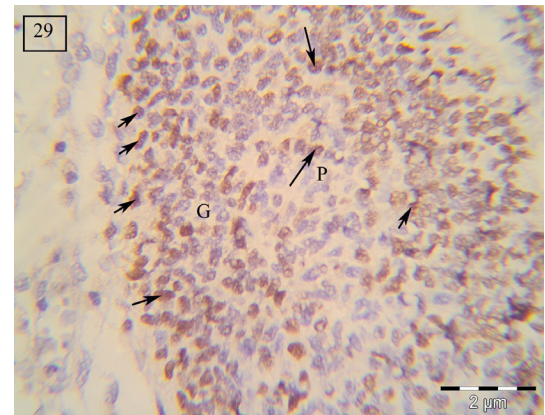


Fig. 29: A photomicrograph of a section in the hippocampus of the neonates of group (III) treated with MSG showed a strong positive Caspase-3 immunoreactive cells (arrows) in the inner granular layer (G) and polymorphic layer (P) of the dentate gyrus region. (Hx. & E.; X 400. Scale bar = 2 μm).

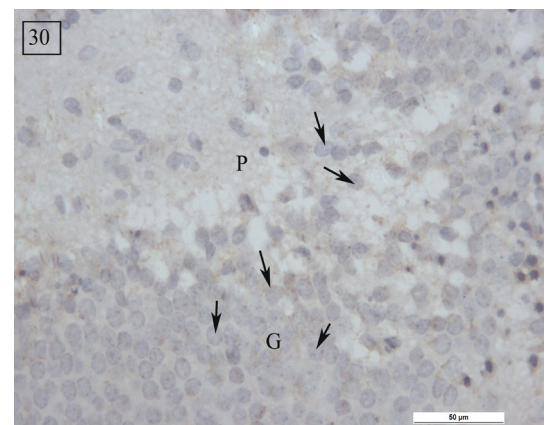


Fig. 30: A photomicrograph of a section in the hippocampus of the control mothers showed negative caspase-3 cells (arrows) in the granular (G) and polymorphic (P) layers of the dentate gyrus. (Hx. & E.; X 400. Scale bar = 50 μm).

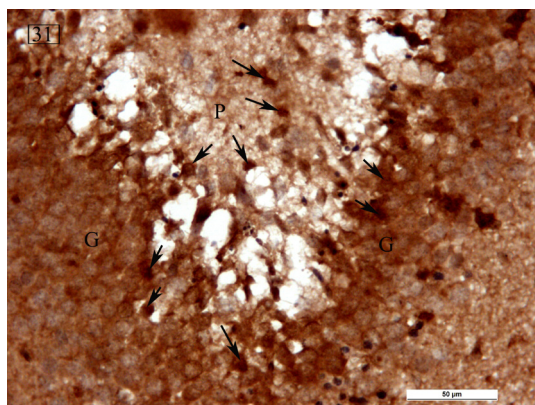


Fig. 31: A photomicrograph of a section in the hippocampus of the MSG treated mothers showing intense increase of caspase-3 positive cells (arrows) and strong staining were observed in the granular(G) and polymorphic(P) layers of the dentate gyrus region. (Hx. & E.; X 400. Scale bar = 50 μm).

Statistical analysis

The statistical analysis of the GFAP immunoeexpression showed a significant increase in the fetuses (group II) and the

neonates (group III) of the MSG-treated animals when compared with the control fetuses and neonates. The statistical analysis of the GFAP immunoeexpression in the MSG-treated mothers (group II) as compared with the control mothers (group I) was significant (Table 1). The statistical analysis of the Caspase-3 immunoeexpression showed a significant increase in the fetuses of (group II) and the neonates of (group III) MSG-treated animals when compared with the control fetuses and neonates. The statistical analysis of the Caspase-3 immunoeexpression in the MSG-treated mothers (group II) as compared with the control mothers was significant. The significant p-value < 0.05 was observed in (fetuses of group II & neonates of group III) and MSG- treated mothers when compared with the control groups (Table 2).

Table 1: Comparison of the area (in %) of the GFAP, among the control groups and the fetuses of the MSG- treated group II, the neonates of the MSG- treated group III and the MSG- treated mothers. SD: Standard deviation, the significant p-value < 0.05 was observed in MSG- treated groups

GFAP test	Mean±SD(control)	Mean±SD (MSG)	t-test	p- value
Fetuses	0.614 ± 0.213	3.397 ± 1.217	14.247	0.0001
Neonates	2.785 ± 1.028	21.335 ± 7.476	7.748	0.0001
Mothers	6.25±2.92	15.38±3.29	16.076	0.0001

Table 2: Comparison of the area (in %) of the caspase-3 among the control groups and the fetuses of the MSG- treated group II, the neonates of the MSG- treated group III and the MSG- treated mothers. SD: Standard deviation, the significant p-value < 0.05 was observed in MSG- treated groups

Caspase-3 test	Mean±SD(control)	Mean±SD (MSG)	t-test	p- value
Fetuses	5.87±2.11	13.26±3.76	13.277	0.000
Neonates	2.310 ± 0.823	34.821 ± 6.121	9.014	0.006
Mothers	3.332±0.821	42.621±3.121	1.113	0.001

DISCUSSION

In the current study, histological examination of the fetuses of the group II (MSG-treated mothers) revealed degenerated neurons and astrocytic hyperplasia (replacement gliosis). The neurons showed abnormal shape and differentiation with Rosenthal fibers and gemistocytes development suggesting the direct toxic effect of MSG intake on the hippocampus of the fetuses due to penetration of MSG into the placental barrier of the mothers. This finding was

in agreement with Yu *et al.*, (1997); and Yu *et al.*, (2006) who reported that monosodium glutamate administration during pregnancy was shown to penetrate the placental barrier and distribute to embryonic tissues, so various developmental histopathological changes in the developing fetal brain were observed following MSG treatment. However, Walker and Lupien, (2000) reported that monosodium glutamate did not readily pass the placental barrier, indicating that the fetus was not exposed to toxic level from the maternal diet through placental transfer as glutamate level in

fetal blood do not rise in parallel with maternal level in rats. In the present study, histological examination of the fetuses of the group II (MSG-treated mothers) showed appearance of gemistocytes. The gemistocytes were enlarged astrocytes which served as phagocytes taking up the degenerating materials thus enlarged in size in the brain injury by MSG. This finding was explained by Szydłowska *et al.*, (2006) who reported that glutamate uptake by astrocytes may prevent excitotoxic glutamate elevation and determine neuronal survival in normal fetuses development. The mechanism by which the astrocytes could regulate extracellular glutamate levels was explained by Hughes *et al.*, (2004) who concluded that glutamate uptake by astrocytes occurred via active uptake through glutamate-transporting proteins, located primarily on astrocytes' membrane which had been shown to reduce the toxic potency of glutamate.

In this study, histological examination of the neonates of the group III (MSG-treated mothers at weaning) provided an evidence for nerve cell damage, hemorrhage in the hippocampus, intense GFAP positive immunoeexpression in the cytoplasm of astrocyte processes and glial scar formation were observed. These findings were confirmed by Rycerz *et al.*, (2014) who investigated alterations in the neurons of hippocampal regions and dentate gyrus in neonate rats treated with MSG. These results provided further support for Beas-Zárate *et al.*, (2001) who reported that histological evidence of neuronal damage, and increased glial cell number were detected mainly in the striatum and hippocampus in the brains of rats treated neonatally with monosodium glutamate. Also, the increased glial cell reactivity in the brain at weaning after neonatal MSG treatment was observed by Lima *et al.*, (2013). However, Walker and Lupien, (2000) reported that monosodium glutamate ingestion during pregnancy was not associated with elevated levels in the maternal milk indicating that the suckling neonate was not exposed to toxic levels from the maternal diet.

The intense GFAP reaction and glial scar formation were explained by Sofroniew and Vinters, (2010) who observed that severe reactive gliosis with glial scar formation associated with an increased regulation of GFAP, marked hypertrophy of cell bodies and processes and marked overlapping of reactive astrocyte processes in the brain injury.

In the current study, histological examination of the mother hippocampus after oral MSG treatment during pregnancy revealed nerve cell degeneration and multicystic encephalomalacia. These findings provided further support for Segura *et al.*, (2006) who concluded that MSG caused an acute necrosis of the neurons in the mothers and fetal rats. Also, the process of neuronal cell death and the elimination of debris by microglia cells proved to be similar in pregnant animals and their fetuses.

These histopathological findings in the pregnant mothers confirmed that the toxic neural effects of MSG were not only restricted to the fetal and neonatal development but also, were affected the adult hippocampus. These neurotoxic effects of MSG in adults were supported by Shivasharan *et al.*, (2013) who reported a significant alteration in hippocampal neuronal histology in adult female Wistar rats treated only for 7 days with intra-peritoneal MSG. Also, Dief *et al.*, (2014) reported that adult wistar rats treated with both oral and subcutaneous MSG for 10 days enhanced neurodegenerative Alzheimer's disease and β -amyloid accumulation in the rat hippocampus.

The cell death in normal developing nervous system is a phenomenon as half of the original cells produced during nervous system development is eliminated by apoptosis to remove unnecessary neurons. The exact mechanism of neuronal cell death induced by toxins, still remains unknown. However, glutamate-induced toxicity can be mediated through apoptosis (Martin *et al.*, 2000). Toxins induced apoptosis through molecular targets. The two main families of apoptotic regulators taken into consideration in mammals are the Bcl-2 family members and the caspases, caspase-3 could promote neuronal differentiation through the activation 38 of signaling pathways (Kelekar and Thompson, 1998). In this study, the caspase-3 immunoeexpression of the fetuses and neonates of the group (II) and the group (III) was significant as compared with the control group (I). These findings were in agreement with Mattson, (2008) who reported that MSG resulted in caspase-3 activation in dendrites of cultured hippocampal neurons as excessive glutamate receptor activation with persistent depolarization producing metabolic and functional exhaustion of the neurons and neural necrosis which was suggesting a role of local caspase-3 activity in

degenerative processes. In conclusion, this study demonstrated that the developing brains of the fetuses and neonatal rats of the treated mothers with MSG are susceptible to pathological injury. My findings indicates the importance of avoiding MSG during pregnancy and lactation periods.

CONFLICT OF INTEREST

None

ETHICAL STATEMENT

The study has been approved by faculty of medicine, Zagazig University Institutional Review Board (ZU - IRB) and performed in accordance with the ethical standards for experimental animal rights.

ACKNOWLEDGMENT

I would like to extend my thanks to Prof. Abd-Elminam Ahmed Ali, Professor of pathology, faculty of medicine, Zagazig University for their help.

REFERENCES

Abass, MA. and El-Haleem, MRA (2011). Evaluation of monosodium glutamate induced neurotoxicity and nephrotoxicity in adult male albino rats. *J Am Sci* 7 (8), 264-276.

Bancroft, J. and M. Gamble (2002). Theory and Practice of histological techniques. 5th ed. Churchill Livingstone, New York London. P: 165-175.

Beas-Zarate, C., S. Rivera-Huizar, A. Martinez-Contreras, A. Feria-Velasco and J Armendariz-Borunda (2001). Changes in NMDA-receptor gene expression are associated with neurotoxicity induced neonatally by glutamate in the rat brain." *Neurochemistry international* 39(1): 1-10.

Bocci, G., A. Fasciani, R. Danesi, P. Viacava, A. R. Genazzani and M. Del Tacca (2001). In-vitro evidence of autocrine secretion of vascular endothelial growth factor by endothelial cells from human placental blood vessels. *Molecular human reproduction* 7(8): 771-777.

Dief, A. E., E. S. Kamha, A. M. Baraka and A. K. Elshorbagy (2014). Monosodium glutamate

neurotoxicity increases beta amyloid in the rat hippocampus: a potential role for cyclic AMP protein kinase. *Neurotoxicology* 42: 76-82.

Giffard, R. G. and R. A. Swanson (2005). Ischemia-induced programmed cell death in astrocytes. *Glia* 50(4): 299-306.

Gudiño-Cabrera, G., M. E. Ureña-Guerrero, M. C. Rivera-Cervantes, A. I. Feria-Velasco and C. Beas-Zárate (2014). Excitotoxicity Triggered by Neonatal Monosodium Glutamate Treatment and Blood–Brain Barrier Function. *Archives of medical research* 45(8): 653-659.

Herrmann JE, Imura T, Song B, Qi J, Ao Y, Nguyen TK, Korsak RA, Takeda K, Akira S, Sofroniew MV (2008). STAT3 is acritical regulator of astrogliosis and scar formation after spinal cord injury. *J Neurosci* 28:7231–7243

Hermanussen, M. and J. Tresguerres (2005). How much glutamate is toxic in pediatric parenteral nutrition? *Acta Paediatrica* 94(1): 16-19.

Higashino, H., A. Niwa, T. Satou, Y. Ohta, S. Hashimoto, M. Tabuchi and K. oshima (2009). Immunohistochemical analysis of brain lesions using S100B and glial fibrillary acidic protein antibodies in a rundic acid-(ONO-2506) treated stroke-prone spontaneously hypertensive rats. *Journal of neural transmission* 116(10): 1209-1219.

Hughes, E. G., Maguire, J. L. McMinn, M. T. Scholz, R. E. and Sutherland, M. L. (2004). Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. *Molecular brain research* 124(2): 114-123.

Kelekar, A. and Thompson, C.B. (1998). Bcl-2 family proteins: the role of the BH3 domain in apoptosis". *Trends Cell Biol.*, 8:324-30.

Kiernan, J. A. (2000). Histological and histochemical methods: theory and practice. 3rd ed. Oxford, Boston, Johannesburg and New Delhi.

Lima, C. B., G. d. S. F. Soares, S. M. Vitor, B. Castellano and R. C. A. Guedes (2013). Neonatal treatment with monosodium glutamate lastingly facilitates spreading depression in the rat cortex. *Life sciences* 93(9): 388-392.

- López-Pérez, S. J., M. E. Ureña-Guerrero and A. Morales-Villagrán (2010).** Monosodium glutamate neonatal treatment as a seizure and excitotoxic model Brain research 1317: 246-256.
- Martin LJ, Siebber FE and Traystam RJ. (2000).** Apoptosis and necrosis occur in separate neuronal populations in hippocampus and cerebellum after ischemia and are associated with differential alterations in metabotropic glutamate receptor signaling pathways. J Cereb Blood Flow Metab., 20: 153-167.
- Mattson MP (2008).** Glutamate and neurotrophic factors in neuronal plasticity and disease. Ann N Y Acad Sci 1144:97–112.
- Meldrum, B. (1993).** Amino acids as dietary excitotoxins: a contribution to understanding neurodegenerative disorders. Brain research reviews 18(3): 293-314.
- Rycerz, K., A. Krawczyk, J. Jaworska-Adamu and I. Krawczyk-Marc (2014).** Effects of monosodium glutamate treatment on calretinin-immunoreactive neurons in hippocampus of postnatal rats. Folia Histochemica et Cytobiologica 52(4): 281-288
- Sanabria, E., Pereira, M. Dolnikoff, M. Andrade, I. Ferreira, A. Cavalheiro, E and Fernandes, M (2002).** Deficit in hippocampal long-term potentiation in monosodium glutamate-treated rats. Brain research bulletin 59(1): 47-51.
- Sant’Diniz, Y., Faine, L. A. Galhardi, C. M. Rodrigues, H. G. Ebaid, G. X. Burneiko, R. C. Cicogna, A. C. and Novelli, E. L. (2005).** Monosodium glutamate in standard and high-fiber diets: metabolic syndrome and oxidative stress in rats. Nutrition, 21 (6): 749-755.
- Segura-Torres JE, Chaparro-Huerta V, Rivera-Cervantes MC, Montes- Gonzalez R, Flores-Soto ME, Beas-Zarate C. (2006).** Neuronal cell death due to glutamate excitotoxicity is mediated by p38 activation in the rat cerebral cortex. Neurosci Lett, 403: 233-238.
- Shivasharan, B., Nagakannan, P. Thippeswamy, B. and Veerapur, V. (2013).** Protective effect of Calendula officinalis L. flowers against monosodium glutamate-induced oxidative stress and excitotoxic brain damage in rats. Indian Journal of Clinical Biochemistry, 28(3): 292-298.
- Sofroniew M. V and Vinters H. V(2010).** Astrocytes: biology and pathology. Acta Neuropathol 119:7–35.
- Szydłowska, K., Zawadzka, M. and Kaminska, B. (2006).** "Neuroprotectant FK506 inhibits glutamate-induced apoptosis of astrocytes in vitro and in vivo." Journal of neurochemistry 99(3): 965-975.
- Tracy, A. L., Jarrard, L. E. and Davidson, T. L. (2001).** The hippocampus and motivation revisited: appetite and activity." Behavioral brain research 127(1): 13-23.
- Veni, N. K., Karthika, D. Devi, M. S. Rubini, M. Vishalini, M. and Pradeepa, Y. (2010).** Analysis of monosodium l-glutamate in food products by high-performance thin layer chromatography." Journal of Young Pharmacists, 2(3): 297-300.
- Walker, R. & Lupien, J. R. (2000).** The Safety Evaluation of Monosodium Glutamate," Journal of Nutrition, 130(4S Suppl), 1049S-52S.
- Weil, Z. M., Norman, G. J. DeVries, A. C. and Nelson, R. J. (2008).** The injured nervous system: a Darwinian perspective." Progress in neurobiology, 86(1): 48-59.
- Yamada, M., Clark, J. McClelland, C. Capaldo, E. Ray, A. and Iulianella, A. (2015).** Cux2 activity defines a subpopulation of perinatal neurogenic progenitors in the hippocampus. Hippocampus 25(2): 253-267.
- Yang WH, Drouin MA, Herbert M, Mao Y, Karsh J (1997).** The monosodium glutamate symptom complex: assessment in a double blind placebo-controlled, randomised study. J. Allergy Clin. Immunol, 99:757-762.
- Yu, T.; Zhao, Y.; Shi, W.; Ma, R, and Yu, L. (1997).** Effects of maternal oral administration of monosodium glutamate at a late stage of pregnancy on developing mouse fetal brain. Brain Res, 747 (2) : 195–206.
- Yu, L., Zhang, Y. Ma, R. Bao, L. Fang, J. and Yu, T. (2006).** Potent protection of ferulic acid against excitotoxic effects of maternal intragastric administration of monosodium glutamate at a late stage of pregnancy on developing mouse fetal brain European Neuropsychopharmacology, 16 (3): 170-177.

آثار الجلوتامات أحادية الصوديوم على تكوين الحصين في الجرذان البيضاء والامهات الحوامل

أسماء محمد طلبية محمد عبدالعال

قسم التشريخ الادمي والأجنة, كلية الطب, جامعة الزقازيق

ملخص البحث

الجلوتامات أحادية الصوديوم هي واحدة من النكهات التي تستخدمها مختلف الصناعات الغذائية وقد تم الإهتمام باستخدام الجلوتامات أحادية الصوديوم بسبب الآثار السامة التي تسببها في المخ خاصة أن في كثير من البلدان لا توجد قيود على كمية الجلوتامات أحادية الصوديوم التي تضاف إلى الطعام لذلك تهدف هذه الدراسة لفحص التغيرات التكوينية المحتملة التي قد تحدث في حصين الجرذان البيضاء في مراحل عمرية قبل الولادة و بعد الولادة بعد تعرض الأمهات إلى الجلوتامات أحادية الصوديوم أثناء الحمل والرضاعة وأيضاً دراسة تأثير الجلوتامات أحادية الصوديوم علي الخلايا العصبية في حصين الأمهات الحوامل لأن الحصين هو منطقة من المخ يتم إنشاؤها من خلايا عصبية جديدة بشكل مستمر طوال الحياة وهو منطقة تتأثر بالتعرض الي الجلوتامات أحادية الصوديوم . وقد أجرى البحث على ثمانون فأراً يتراوح اوزانهم ما بين مائتي إلى مائتي وعشرون جرام لكل جرذا (ستون أنثى وعشرون ذكراً) وبعد التزاوج في بيت الحيوان كلية الطب جامعة الزقازيق تم تحديد اليوم الأول للحمل عن طريق الكشف المهبلي ثم قسمت الفئران الحوامل إلى ثلاث مجموعات على أقفاص متعددة كل مجموعة تحتوي علي عشرين جرذا. المجموعة الأولى هي المجموعة الضابطة والمجموعه الثانيه هي مجموعه معالجه بالجلوتامات أحادية الصوديوم حتى اليوم التاسع عشر من الحمل بجرعة ثمانمائة وثلاثون جرام لكل كيلو جرام من وزن الجسم والمجموعه الثالثه هي مجموعه معالجه بنفس جرعة الجلوتامات أحادية الصوديوم حتى نهاية الحمل وأثناء أرضاع ثلاثون فأراً حديثي الولادة لمدة ثلاثة أسابيع وقد وجد عند الفحص بالميكروسكوب الضوئي أن هناك تهتك في نسيج الحصين في مرحلة قبل الولادة وظهور نزيف بالحصين في مرحلة بعد الولادة نتيجة تعرض الأمهات للجلوتامات أحادية الصوديوم كما لوحظ فراغات واسعة حول الخلايا العصبية في جميع أجزاء الحصين خاصة التليف المسنن كما لوحظ في المجموعات المعالجه زيادة في درجة صباغة الخلايا النجمية بصبغها بمضادات الشعيرات الخيطية البروتينية الحامضية للخلايا النجمية ووجود تفاعل مناعى قوى للكسبيز في خلايا الحصين.

لذلك من هذه النتائج يتضح ان الجلوتامات أحادية الصوديوم لها آثار سمية عصبية قوية على نمو الحصين للأجنة وحديثي الولادة وأيضاً حصين الأمهات باستخدام الدراسات الهستوكيميائية المناعية والمرفومترية وينبغي تجنب تناول الأطعمة التي تحتوى على الجلوتامات أحادية الصوديوم خاصة في فترات الحمل والرضاعة.