The effects of Aspartame on the postnatal development of the cerebellum in male albino rat offspring

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Abstract

Background: Aspartame (ASP) is a non-nutritive sweetener that is greatly used in drinks, foods, beverages and pharmaceuticals**.** There are controversies about its safety. So, studies are important to prove or disapprove the present fears about aspartame.
Objectives: It is to evaluate the effect of ASP on the postnatal development of cerebellum in rat offspring.
Material and Methods: Eight male offspring of each control and experimental groups were randomly selected at the postnatal day (PND): 1,7,15.21 and 60 days. Aspartame (250 mg/kg body weight/day) was given orally to experimental group of animals during pregnancy and lactation period. Specimens were prepared for light and electron microscopic studies. Morphometric and statistical studies were done to measure the diameter of granular cells nuclei.
Results: Control group of animals showed normal development of cerebellar cortex. Cerebella of the experimental group of animals showed a disrupted architecture with degenerative changes especially in Purkinje cells. Splitting and disruption of the myelin sheath were detected. Experimental group showed a highly significant decrease in the mean granular cell nuclear diameter ($p<0.001$) when compared to the control group.
Conclusions: Administration of aspartame during pregnancy has harmful effects on the developing cerebellum of albino rat's offspring.
Keywords: Cerebellum, Postnatal, Aspartame.

Introduction

Aspartame (ASP) is an artificial sweetener that is used in wide range of many foods, drinks, pharmaceuticals and beverages **(Rencuzogullari al.,2004).** After ASP ingestion, it is splittedinto methanol, aspartic acid and phenylalanine **(Humphries et al.,2008).**Phenylalanine crosses to the brain and Aspartic acid is considered the precursor for some amino acids as glutamate and glutamine. The excess of them, especially glutamate, increases excitability of neurons that induce death of astrocytes and neurons**(Pinheiro and Mulle, 2008)**.The liver metabolizes methanol to formaldehyde. Methyl alcohol leads to depression of the central nervous system (CNS)**(Butchko et al.,2002).**Another ASP metabolite that is formed as a consequence of extended periods of storage of food products is the diketopiperazine(DKP). Recently, it is considered that DKP isa highly carcinogenic factor **(Soffrittiet al.,2006)**.

Some investigators suggested that ASP use leads to increase the incidence of some tumors as leukemia, lymphoma, urinary tract tumors, and neurological tumors **(Soffritti et al.,2010**).Also, ASP consumption was associated with increase in the susceptibility for type 2 diabetes (**Nadipelly et al., 2017),**preterm delivery **(Englund-Ögge et**

al.,2012), nephrotoxicity **(Saleh ,2014)** and hepatotoxicity **(El Haliem and Mohamed , 2011)**.This study investigated the effect of ASP on postnatal development of the cerebellum.

Materials and Methods

Animals and Experimental design

Thirty adult albino female rats and 10 adult male albino rats aged about 3 months and weighing about 170–250g were gained from the Animal House, Faculty of Medicine, South Valley University were used in this study. Wistar-albino rats were preserved in hygienic conditions, homed in standard cages, fed *ad libitum* and all have open access to water.

Mating the animals and detection of pregnancy

Every three female rats were mated with one male rat overnight. Next morning, vaginal smear was done to detect the presence of sperms. The female rats were considered pregnant if there was a copulatory plug (semen) or sperms in the vaginal smears. This is the gestational age 0(**Ognio et al., 2003**)

The pregnant rats were separated into two equal groups,Control group (n =15) and experimental group (n =15).Pregnant mothers of the experimental group received 250mgAsp/kg/day dissolved in distilled water orally by intra-gastric intubation for6 weeks (the duration of pregnancy and lactation). The mothers of the control group received only distilled water on the same regimen.

Eight male offspring of each group(control group and experimental) were randomly selected at each of the following ages: 1, 7, 15, 21 and 60 days. The animals were sacrificed and transcardially perfused with physiological saline.

For Light Microscopic Study

Cerebella were removed and placed in Bouin's solution for 24 hours for fixation. Specimens were put in ascending grades of ethanol, filtered, dipped in paraffin and then sectioned serially in the sagittal plane at 5 µm **(Bancroft and Gamble, 2002).** Hematoxylin and Eosin (H&E) stain was used for staining of the sections.

Examination of the sections was done using Leica Microscope, CMSGmbH, Wetzlar, Germany microscope at the Human Anatomy and Embryology Department, Faculty of Medicine, South Valley University.

For Electron Microscopic Study

The cerebella at the postnatal days 21 and 60 were processed for semithin and ultrathin section. The animals were transcardially perfused with isotonic saline followed by 5% glutaraldehyde. The cerebella were dissected in the same fixative, cut into small pieces about 2 mm thick and post fixed in osmium tetroxide at concentration of 1%.The samples were put in ascending grades of ethanol and dipped in Epon resin. Semi-thin sections (1µm) were cut using a glass knife in the ultramicrotome. Toluidine blue stain was used for staining the semithin sections. Other ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate **(Bancroft and Stevens, 1996).**

Examination and photographing of the specimens were done using JEM 1200 EXII Transmission Electron Microscope in South Valley University.

Chemicals: ASP in the form of powder was gained from Al-Haditha Pharma Company. This used dose of ASP is similar to the human dose, that is ranging from 40-50 mg/ kg body weight per day because the rat metabolizes ASP faster than human(**Abd El-Samad, 2010**)

Morphometric and statistical analysis

The diameter of granule cell nuclei at the postnatal days 21 and60 of both control and ASP treated groups were measured (at magnification of400Microscope). The previous parameter was

measured using the software (ImageJ).Variables were represented by mean ± standard deviation (Mean ± SD). Data analysis was done by using t-test and one-way ANOVA test of SPSS 22to calculate p value. The measurements were considered significant if $p < 0.05$.

Results

Light microscopic result

The cortex of the cerebellum of the control group at PND1 formed of: the external granular layer(EGL)that lie beneath the pia matter, then the molecular layer (ML) and the last layer is the internal granular layer (IGL). The EGL composed of four to five layers of oval or rounded cells. The ML appeared as a pale zone deep to the external granular layer and contained Purkinje cells that were coordinated in multiple rows. The Purkinje cells were characterized by having an apical cone, pointing towards the surface of the cerebellar cortex. The IGL had densely stained cells of variable shape and size. The white matter was located deep to the IGL and it was filled with less packed oval or fusiform cells (Fig.2A).

Effect of ASP on the cerebellum at PND1 of experimental group showed reduction in the EGL thickness in comparison with the control group. The Purkinjie cells showed disruption in the ML and some of them were detected among the granular cells (Fig.2B).

The cerebellar cortex of the control group at PND7 appeared to have four layers: The outer layer was the EGL that covered the surface of the developing cerebellum and its thickness was increased in size when compared with the age of one day old animal, the 2nd layer was the ML that appeared as an immature thin layer with a few number of cells.

The next layer was the Purkinje cell layer, its cells tend to arrange in one row. The following layer was the IGL that was thick layer and stuffed with small cells called granule cells, which had large nuclei and a little amount of cytoplasm. The white matter appeared as immature layer and contained densely stained oval or rounded cells (Fig.3A).

However, examination of the cerebellum at PND7 of experimental group revealed apparent reduction in the thickness of the EGL and ML and disturbance in the linear organization of the Purkinje cell layer. Most cells of IGL became vacuolated. Vacuolations were present in the internal granular layer and also in the white matter. Congestion of the blood vessels was present between the cerebellar folia (Fig.3B).

Histological examination of cerebellar sections in control group at PND15 showed reduction of the thickness of the EGL in comparison with the PND7. The ML contained a small number of cells that markedly increased in size. The Purkinje cell layer had large oval cells arranged in single row between the upper ML and the IGL. Their nuclei were large, vesicular with pale cytoplasm. The IGL were small and deeply stained. The white matter appeared mature (Fig.4A).

However, examination of the cerebellum of the experimental group at PND15 showed a reduction in the thickness of EGL in comparison with the control of the same age. There were deformed shrunken and deeply stained Purkinjie cells. Halos of empty spaces appeared around purkinjie cells with condensed nuclei. Cavitations appeared in the ML and white matter as a sign of necrosis (Fig.4B).

Examination of the cerebellum of the control group at PND21 revealed that the cerebellar cortex became well developed, like the adult architecture. There was marked increase in its size and the fissures were increased in depth. The EGL became very thin and might be absent in some areas. The ML appeared well developed and contained mainly fibers and few cells. The

Purkinjie cell layer contained one row of cells that are oval shaped with an apical dendrite. The granular layer appeared mature with densely populated granule cells (Fig.5A).

Experimental group at PND21 showed that the EGL was very thin and disappeared in some areas, there was deformed Purkinjie cells with condensation of their nuclei. Some of them were surrounded by empty halo and the granular cells had some pyknotic nuclei (Fig.5B).

Histological examinations of H&E stained sections of the control group atPND60 showed the normal architecture of adult cerebellum. The outer layer was the ML that consisted mainly of fibers and few cells. The next layer was Purkinjie cell layer that had one row of purkinjie cells that appeared oval in shape with its apical dendrite extended to the ML. The granular layer consisted of densely stained closely packed granule cells. In between these cells there were the cerebellar islands (Fig.6A).

Experimental group at PND60 showed degeneration of Purkinjie cells in some areas and some of them were deeply stained with non-identified nuclei. Cavitations were present in the ML and in the white matter as a sign of necrosis (Fig.6B).

Electron microscopic results

Examination of section of the cerebellum of control group at PND21showed normal appearance of Purkinje cell with large euchromatic nucleus and prominent nucleolus. The cytoplasm has Golgi bodies, rough endoplasmic reticulum and mitochondria (Fig.7A).

Experimental group at PND21 showed shrunken Purkinje cell that associated with electron dense cytoplasm. The nuclear envelope appeared irregular with eccentric nucleolus. The cytoplasm had many vacuoles. Some of granule cells appeared with dense clumping of the chromatin. The Bergman astrocyte had many vacuoles (Fig.7B).

Examination of sections of the cerebellum of control group at PND21revealed that the granular cells appeared oval or rounded in shape, their nuclei were large and contains heterochromatin. They contained a little amount of cytoplasm (Fig.8A).

Experimental group atPND21showed that the nuclei of granule cells appeared with an increased condensation of their heterochromatin. Some cells showed shrinkage of their nuclei with electron dense cytoplasm. Some myelinated nerve fibers showed splitting in their myelin sheath. The Bergman astrocytes appeared surrounding part of the Purkinjie cell. Some astrocytes contained small spherical mitochondria; another astrocyte contained a clumped chromatin with an electron dense cytoplasm. The surrounding tissue contained many vacuoles (Fig.8B).

Examination of sections of the cerebellum of controlgroup atPND60 showed that the granule cells were arranged in groups. They had rounded or oval nuclei, peripherally clumped chromatin and thin film of cytoplasm. Oligodendrocytes were present between the granule cells. These cells appeared as large irregular cells that contain large nuclei. Their processes extended to reach and cover the granular cells (Fig.9A).

Experimental group atPND60 showed that the granular cells appeared with dense clumping of their chromatin, irregular nuclear envelope and cytoplasmic vacuoles. There were many vacuoles in the extracellular space (Fig.9B).

Examination of section of the cerebellum of control group atPND60 showed normal Purkinje cell with large euchromatic nucleus. The cytoplasm contained Golgi bodies, rough endoplasmic reticulum and mitochondria(Fig.10A).

Experimental group atPND60 showed a very shrunken Purkinje cell with an electron dense

cytoplasm. There was area of degeneration and many areas of vacuolationsin the surrounding tissue (Fig.10B)

Morphometric Results

Mean granular cell nuclear diameter in experimental group at PND 21 showed a moderately significant decrease in its length when compared to the control group P<0.01(table 1).

Table (1) shows Mean ± SD of the diameter of granular cells at PND21.

Variables	Control group	Experimental group	p-value
Nuclear diameter of granule cells.	4.0948±.67915	3.6533±.68972	p<0.001

Significance probability: p<0.05 * Significant, p<0.01** Moderately significant, p<0.001 ***Highly significant

Mean granular cell nuclear diameter in experimental group at PND 60 showed highly significant decrease in its length when compared to control group p<0.001(table 2).

Table (2): Mean ± SD of the diameter of granular cells at PND60.

Variables	Control group	Experimental group	p-value
Nuclear diameter of granule cells.	5.8239±1.27863	3.9866±.75180	p<.000

Significance probability: p<0.05 * Significant, p<0.01** Moderately significant, p<0.001 ***Highly significant

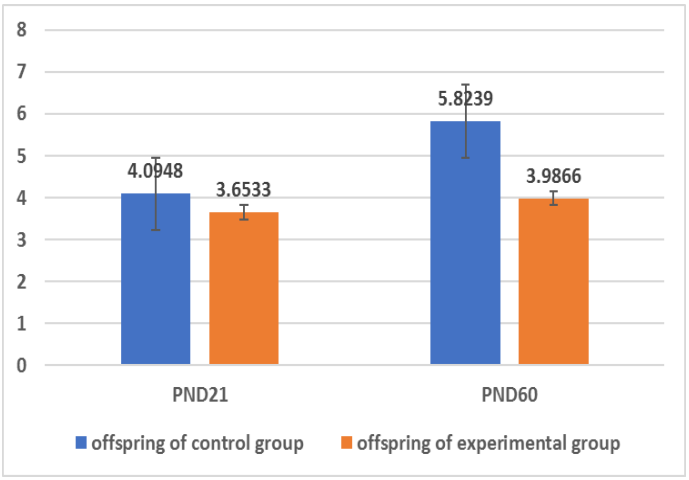
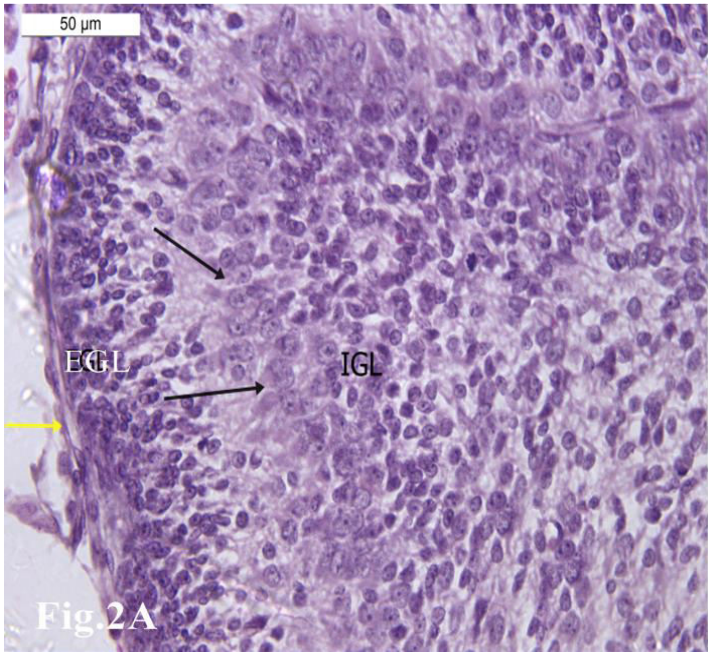


Figure 1: Histogram shows the mean diameter of granular cells at PND21 &PND60 in both groups in a constant surface area (1861.754) micron².



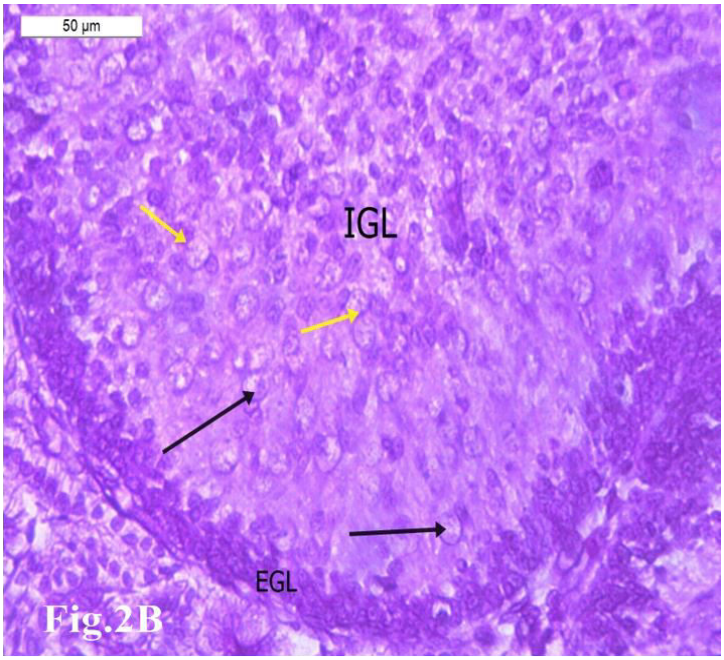
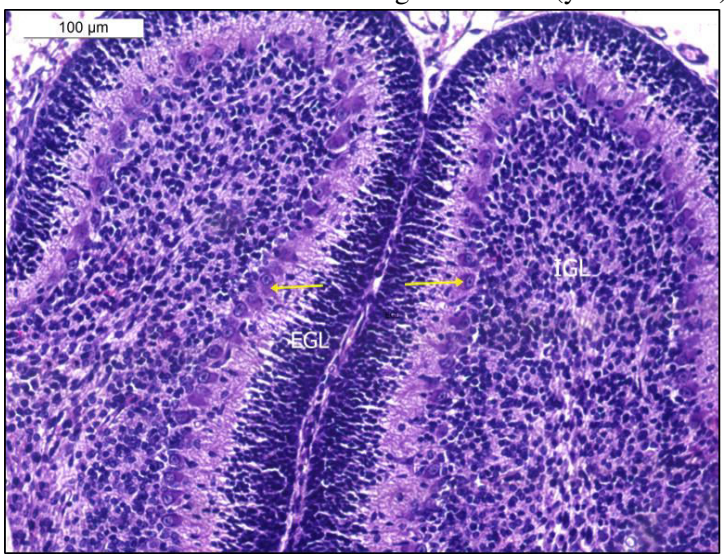


Figure 2A: A photomicrograph of section of a cerebellar rat of the control group at PND1 showing that the cerebellum is composed of thick mitotically active external granular layer(EGL) that lie beneath the pia matter(yellow arrow). Purkinjie cells (black arrow) are arranged in multiple layers. The inner granular lie internal to the purkinjie cells **(H&E, x400).2B**: A photomicrograph of section of a cerebellar rat of theexperimentalgroup at PND1 showing that the (EGL) decreased in its thicknes.The Purkinjie cells(black arrow)show an irregular dispersion in the molecular layer & some of them are detected in-between the granular cells(yellow arrow). There is decreased density of the IGL**(H&E, x400)**.



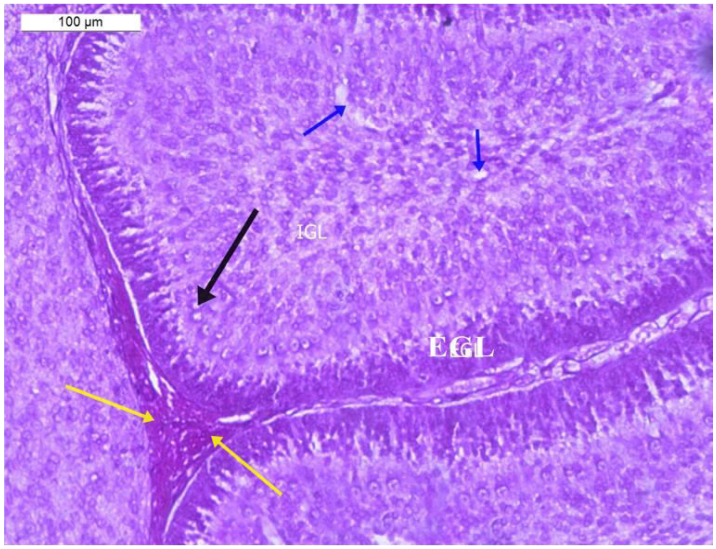
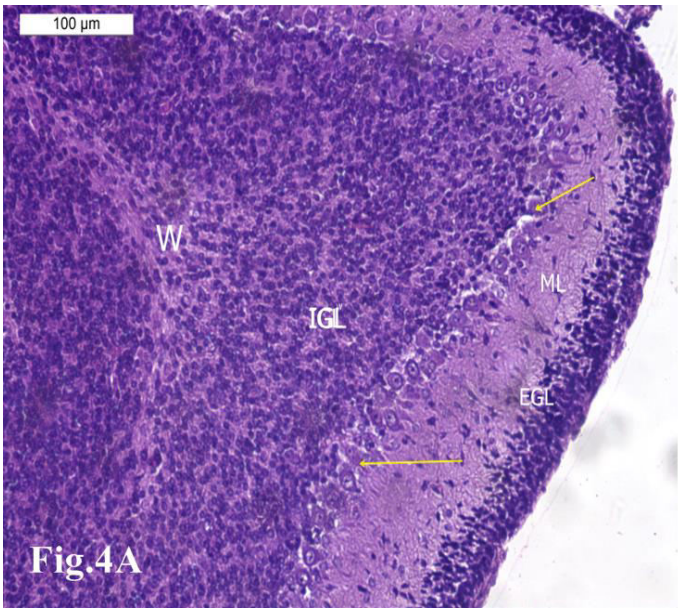


Figure 3A: A photomicrograph of a section of a cerebellar rat of the control group at PND7 showing that the EGL is thicker than in PND1.TheML contains few cells. The Purkinjie cells (yellow arrow) tend to arrange in one layer. The IGL is composed of deeply stained many granule cells. The white matter appears immature and contains rounded cells **(H&E, x200).3B**: A photomicrograph of a section of a cerebellar rat of the experimental group at PND7showing an apparent reduction of the thickness of the EGL and apparent decrease in the thickness of the ML. Disturbance in the linear organization of the Purkinje layer (black arrow). Most cells of the IGL become vacuolated. Vacuolations in the internal granular layer (blue arrow) is observed. There is a congestion of the blood vessels between the cerebellar folia (yellow arrow)**(H&E, x200).**



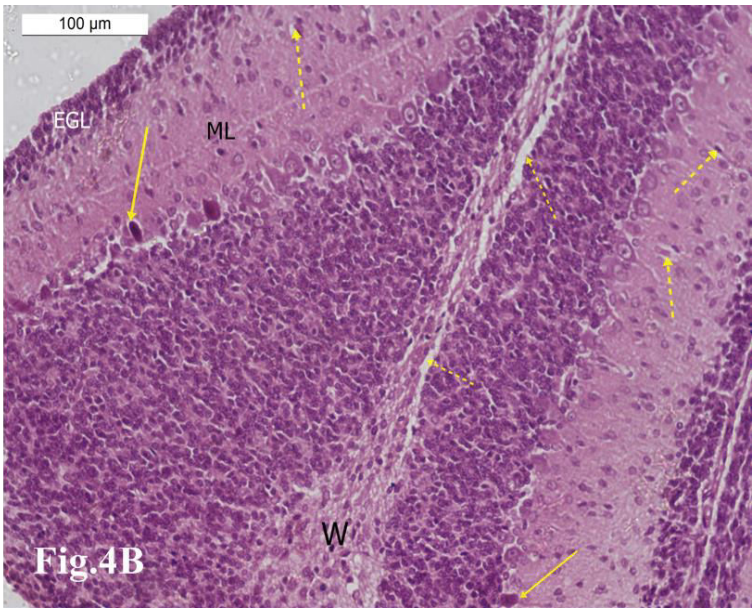
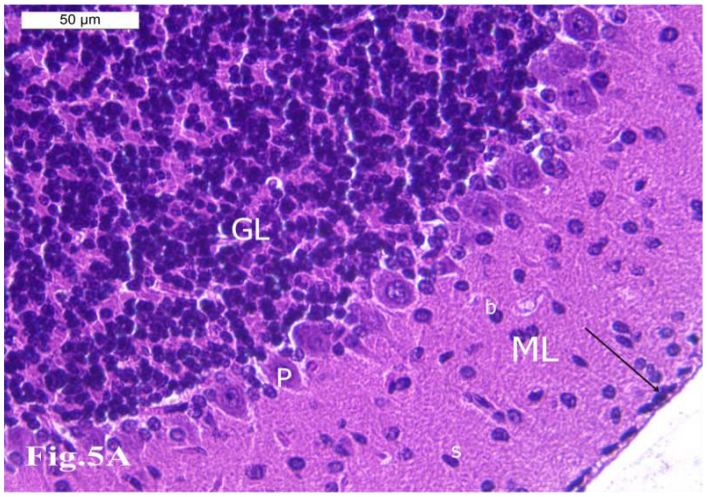


Figure 4A: A photomicrograph of section of arat cerebellum of the controlgroup at PND 15.There is a reduction in the EGL . The ML is well developed ,markedly increased in thickness and contain few cells. The Purkinjie cells (arrow)are in single raw. The density of neurons and thickness of granular layer(IGL) are increased when compared to PND7.The white matter (W) begins to appear **(H&E, x200). 4B**: A photomicrograph of section of arat cerebellum of the experimental group atPND15 showing deformed , shrunken and deeply stained purkinjie cells. Halos of empty spaces appear around Purkinjie cells with condensed nuclei (yellow arrow). There is a reduction in the EGL thickness in comparison with the control. Vacuolations appear in the ML and in the white matter(W)(dotted arrow) **(H&E, x200)**



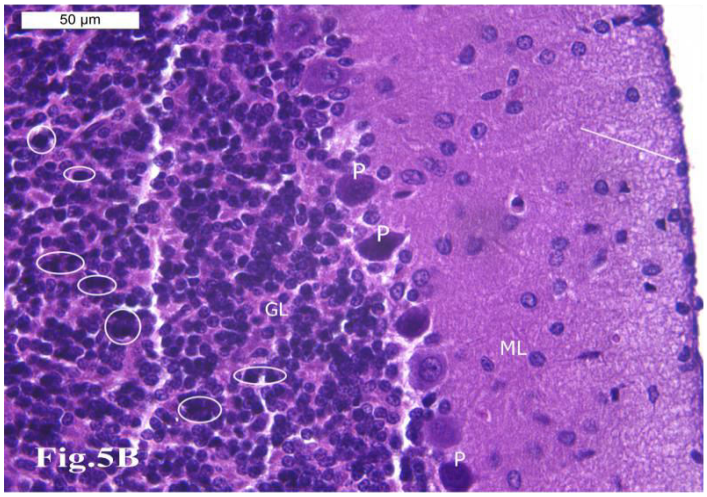


Figure5A: A photomicrograph of a section in a rat cerebellum of thecontrol groupatPND21 showing the EGL (black arrow)that becomes very thin and may be absent in some areas.The ML appears well developed that contains mainly fibers and few cells.The Purkinjie cell layer (P)contains one row of cells that are oval in shape with an apical dendrite.The granular layer (GL) appears mature with densly populated granule cells**(H&E, x400).5B**: A photomicrograph of a section in a rat cerebellum of the experimental group at PND21 showing the external granular layer (arrow)is very thin and disappears in some areas , deformed Purkinjie cells with condensation of their nuclei(p)and some of them are surrounded by empty halo and the granular cells has some pyknotic nuclei(circle)**(H&E, x400).**

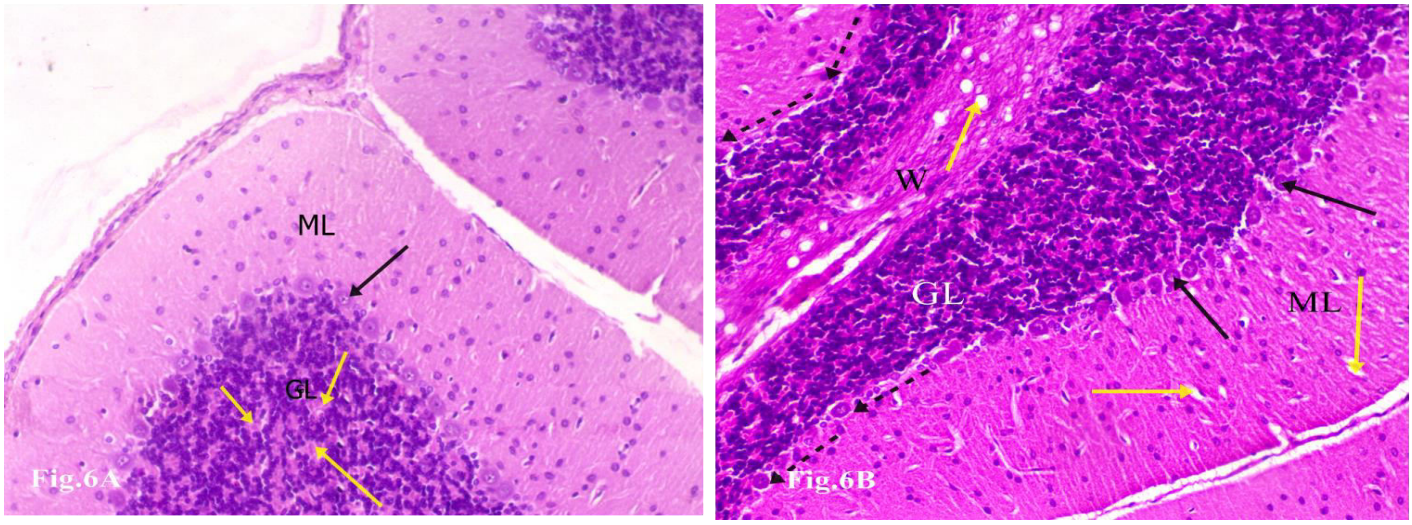


Figure 6A: A photomicrograph of a section in a rat cerebellum of the controlgroup at PND60 showing the normal architecture of adult cerebellum. The outer layer is the ML that contains mainly fibers and few cells. The next layer is Purkinjie cell layer (black arrow) that consists of one raw of Purkinjie cells that appear oval in shape with its apical dendrite that extends to ML. The granular layer (GL) consists of densely stained closely packed granule cells. Notice the cerebellar islands(yellow arrow)in between granular cells **(H&E, x200).6B**:A photomicrograph of a section in a rat cerebellum of the experimental group at PND60 showing many vacuoles in the ML and white matter(W) (yellow arrow).The Purkinjie cell layer showing degeneration in some areas(dotted arrow)and some of them are deeply stained(black arrow)with non-identified nuclei**(H&E, x200).**

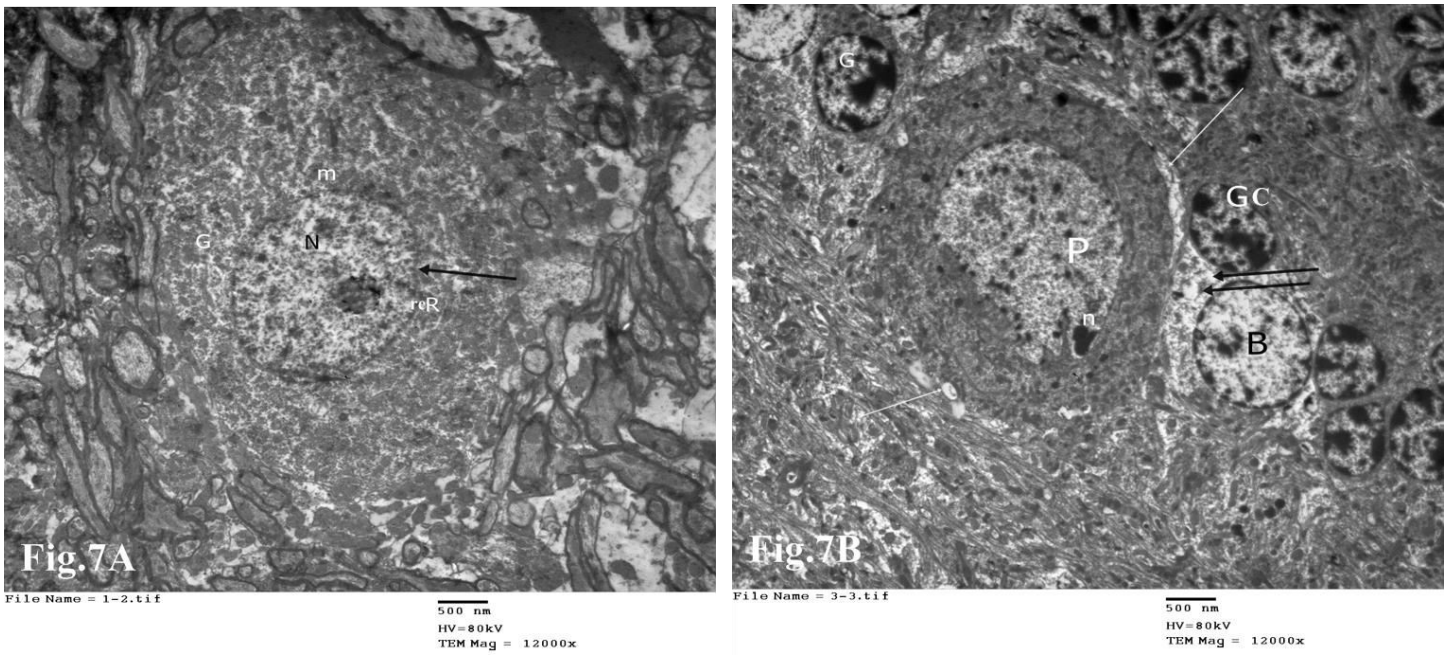
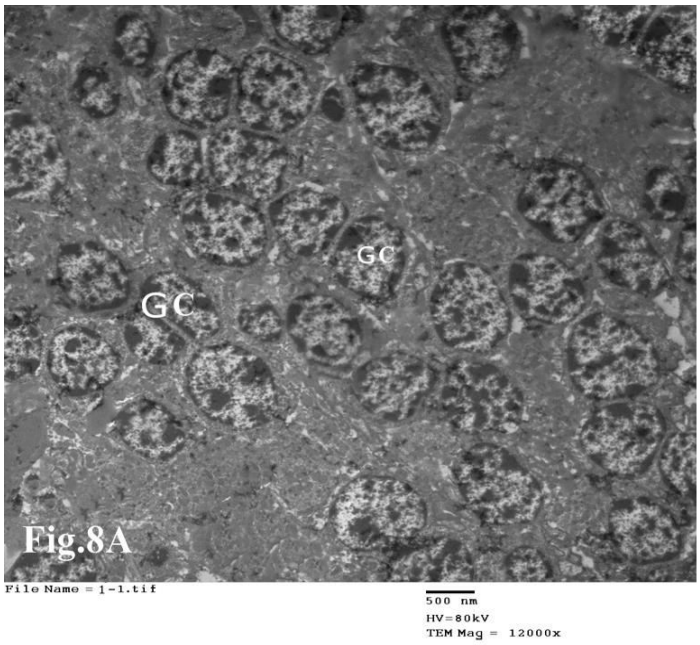


Figure 7A: An electron micrograph of a section in arat cerebellar cortex of the control group atPND21 showing a normal appearance of Purkinje cell with large euchromatic nucleus(N) and apparent nucleolus. The cytoplasm contains Golgi bodies (G), rough endoplasmic reticulum (reR)and numerous mitochondria (m).7B: An electron micrograph of section of a rat cerebellum of the experimental group atPND21 showing shrunken Purkinje cell (P)associated with electron dense cytoplasm. Its nuclear envelope appears irregular with eccentric nucleolus(n). The cytoplasm contains many vacuoles(white arrow). Some of the granule cells(GC)appear with dense clumping of their chromatin. The Bergman astrocyte contains vacuoles (black arrow).



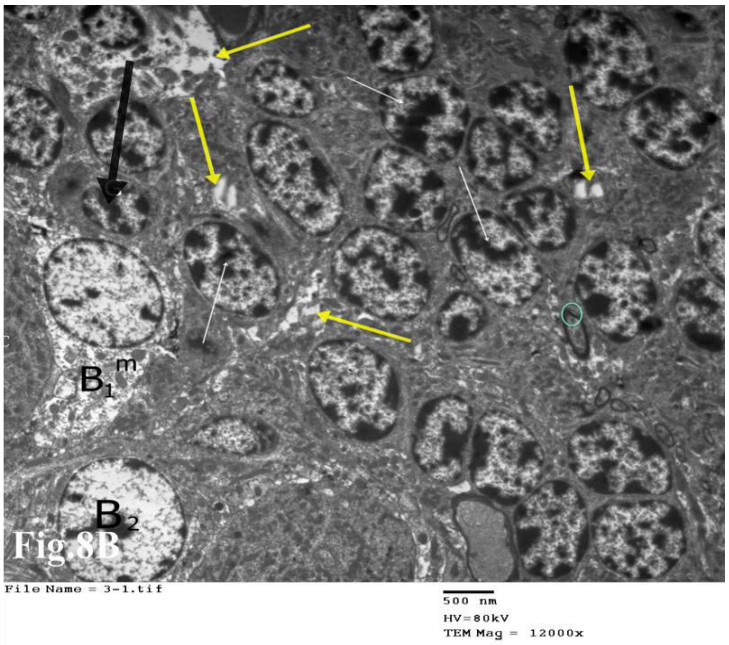
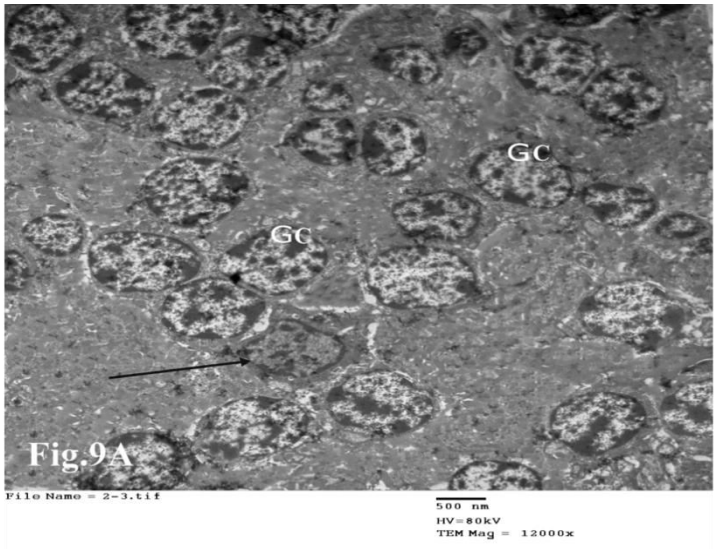


Figure 8A: An electron micrograph of a section in a rat cerebellum of the control group atPND21 showing a group of granular cells (GC). They are oval or rounded in shape. Their nuclei are large and contain heterochromatin. They contain a little amount of cytoplasm.**8B**: An electron micrograph of a section in a rat cerebellum of the experimental group atPND21 showing granule cells with increased condensation of their heterochromatin (white arrow). Some granule cells(black arrow) show shrinkage of their nuclei with electron dense cytoplasm. Some myelinated nerve fibers show splitting in myelin sheath(O). The Bergman astrocytes appear surrounding part of the Purkinjie cell. The astrocyte (B1) contains small dense mitochondria(m),the other astrocytes contain a clumped chromatin with an electron dense cytoplasm. The surrounding tissue contains many vacuoles (yellow arrow).



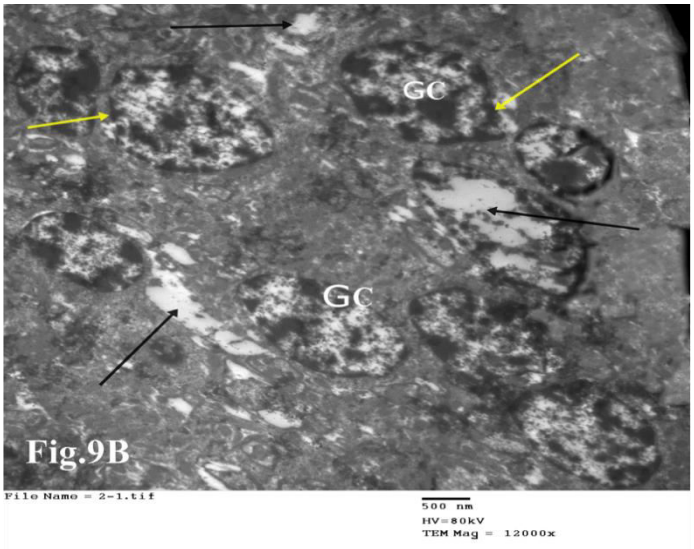
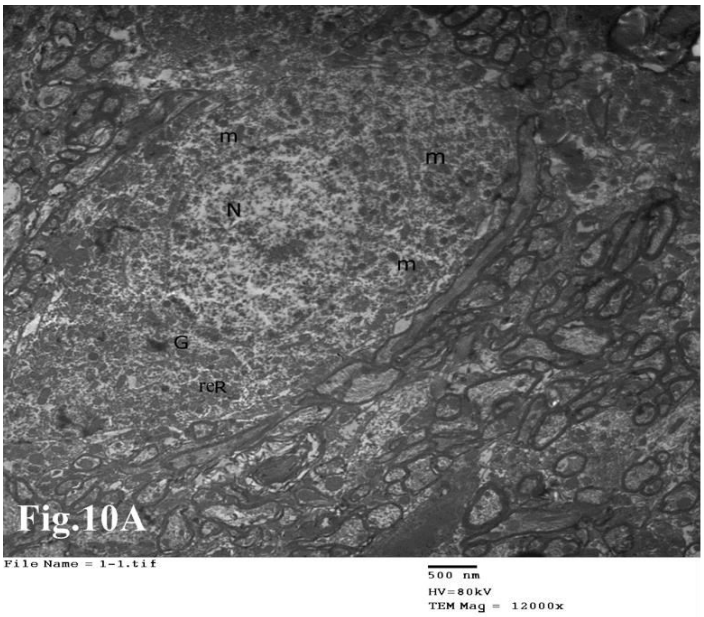


Figure 9A: An electron micrograph of a section of a rat cerebellum of the control group atPND60 showing the granule cells(GC). They arranged in groups. They have rounded or oval nuclei, peripherally clumped chromatin and thin film of cytoplasm. Oligodendrocytes (black arrow) are present between the granule cells.**9B**: An electron micrograph of section of a rat cerebellum of the experimental group atPND60 showing the granular cells(G) with dense clumping of their chromatin, irregular nuclear envelope and cytoplasmic vacuoles (yellow arrow). There are many vacuoles in the extracellular space (black arrow).



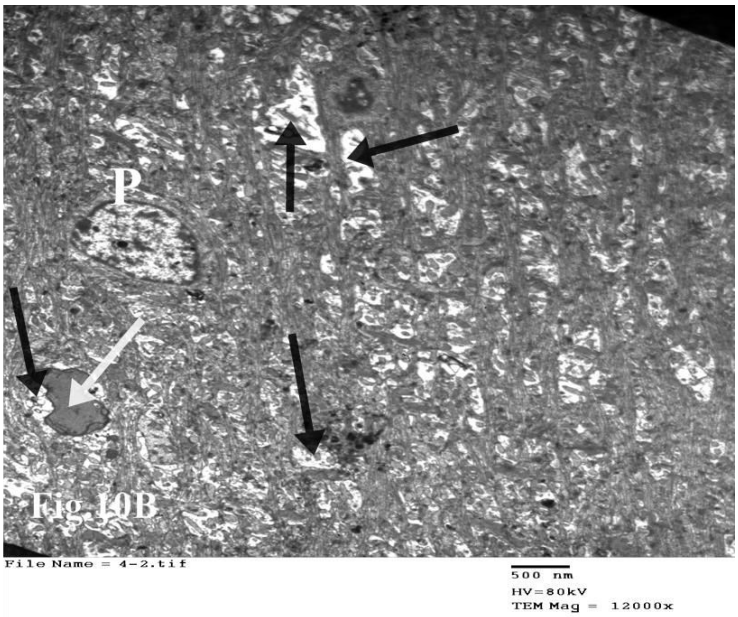


Figure 10A: An electron micrograph of a section of a rat cerebellum of the control group atPND60 showing a normal Purkinje cell having large euchromatic nucleus (N). The cytoplasm is rich in Golgi bodies (G), rough endoplasmic reticulum (reR) and many mitochondria (m).**10B**: An electron micrograph of section of a rat cerebellum of the experimental group atPND60 showing much shrunken Purkinje cell (P) with electron dense cytoplasm. There is area of degeneration (white arrow) and many areas of vacuolations(black arrow)in the surrounding tissue.

Discussion

Artificial sweeteners are considered essential food additives. ASP is extremely used as an artificial sweetener **(Duerfahrt et al., 2003)**. These sweeteners are frequently used in a lot of food products, drugs, and by diabetic patients **(Chatsudthipong and Muanprasat.,2009).**

The cerebellum is highly sensitive organ to the changes that occur during the development in its histological structure. This might be due to maternal exposure to some diseases or certain chemicals (toxicants or drugs) during early pregnancy **(Qin et al.,2006).**The cerebellum undergoes considerable events of maturation and development until it reaches its adult neuronal structure in the first three weeks of postnatal life in the rat**(De-Filippi et al., 2005)**

In the present study, the control group at PND 1showedthatthe cerebellar cortex consisted of three layers: the EGL, the ML and the IGL. The white matter was filled with less packed oval or fusiform cells.

The EGL composed of 3-5 layers. The ML appears as a pale zone superior to the IGL. It contains Purkinje cells that tend to arrange in several irregular rows. The Purkinje cells have an apical cytoplasmic cone, pointing towards the surface of cerebellar cortex. The IGL contains deeply stained cells of different shape and size. The white matter contains packed fusiform or oval cells **(Elsawy et al., 2013).**

Mohamed and Mohamed,(2018) stated that, the ML could be hardly recognized at the second day postnatally.

In the present study, the cerebellar cortex of control group at PND7appeared to have four layers the EGL that increased in size, the 2nd layer is the ML that appears as an immature thin layer with few number of basket cells then the Purkinje cell layer, and finally is the IGL . The whit matter appeared immature layer that contained densely stained oval or rounded cells. Mostly, the basket cells are present at the end of the PND7, while the stellate cells appeared towards the end of the 3rd week(**Ahmed & Eid., 2015**). **Vastaghet al.,(2005)** revealed that the Purkinje cells constitute a monolayer by the PNDP4–P5.

After ASP administration, reduction of the thickness of the EGL occurred when compared to the control group and the Purkinjie cells lost their arrangement.

These findings could be due to that aspartate causes excitation of the cells of the CNS. It causes continuous depolarization at the postsynaptic membrane. This might cause excessive firing of the neurons (**Humpheries et al.,2008).**

The excess glutamate and aspartate in the blood plasma shortly after ingesting ASP lead to increase in their concentration in certain areas of the brain. Their effect in infant rodents could be due to decrease in the ability of the infant's liver and intestinal epithelium to transaminate aspartate and glutamate, or to a minor degree of expression of the glial glutamate transporters in the brain at this developmental stage. These changes of expression can affect excitotoxic phenomena and shares in the pattern of susceptibility in the neonatal rodent (**Meldrum, 2000**).

It is established that the immature brain is sensitive four times than the mature brain for the harmful effects of the excitatory amino acids. Also, it is well known that the placenta accumulates many of these toxic amino acids towards the fetal surface of the placenta **(Battaglia, 2000).**

At PND21 the EGL became very thin and may be absent in some areas. The cerebellar structure resembles that of PND60. There was marked increase in its size and fissures were increased in depth. The ML appeared well developed and contained mainly fibers and few cells (basket cells & stellate cells). The Purkinjie cell layer had one row of cells that are oval shaped with an apical dendrite. The granular layer consisted of granule cells with cerebellar islands in between.

The granular cells migrate inward, to form the IGL. The EGL disappears towards the end of the 3rd week postnatally, and three well-defined layers have formed**(Xu et al., 2013).**

After ASP exposure there was deformed, shrunken and deeply stained Purkinjie cells. Empty spaces surrounding the Purkinje cells were present. Many cavitations appeared in the ML and white matter.

The focal loss of Purkinje cells, their abnormal shape and their increased staining denotes their major affection. They are the chief cells of the cerebellar cortex and they are the only cells that send information from the cerebellum to the outside (**Gartner and Hiatt ,2006**).

The dark neurons in boththe Purkinje and granular layer may be resulted from neuronal degeneration. This degeneration is due to exposure to Asp metabolites that cause disturbance of the blood–brain barrier and destruction of nerve cells(**Mohamed,2013**).

These metabolites reach the neurons across the blood–brain barrier and lead to occurrence of several reactions. The excitotoxins cause excessive stimulation of the nerves till firing. Phenylalanine and aspartate negate the action of the normal enzymes that is required to stop the continuous firing of these nerve cells **(Olney ,1996).**

The perineuronal space observed around the Purkinje cells in Aspartame-treated group could be due to shrinkage of cells and loss of cell processes. **(Sobaniec et al., 2001)**

Vacuolations found mainly in both the white matter and ML of the experimental group can be due to the lack of cellular elements of the tissue or organ(**Eluwa et al., 2013**). These results are in agreement with the previous studies on the cerebellum seen as a result of ASP administration (**Mohamed, 2013**).

In the current study, the ultrastructure of the cerebellum of PND21 and PND60 had many pathological changes due to ASP exposure. The Purkinje cells were shrunken and had electron dense cytoplasm. The nuclear envelope appeared irregular with eccentric nucleolus. The cytoplasm had many vacuoles. The granular cells contained dense clumping of the chromatin, with irregular nuclear envelope and cytoplasmic vacuoles. There were many vacuoles in the extracellular space.

The increased density of the cytoplasm, increased chromatin condensation and distortion of mitochondria are due to direct toxicity on neurons. This toxicity induces major harmful events, as abnormal production of proteins, inhibition of oxidative phosphorylation, and dysfunction in detoxication **(Sobaniec et al., 2001).**

The cytoplasmic vacuoles observed in this study may be induced by Aspartic acid and methanol. They induce amino-acid imbalance in the microenvironment around the neurons. They allow entrance of free radicals. The free radicals produce vacuoles in the cytoplasm by interaction with the lipids and proteins of many organelles. Also, these free radicals could change the nuclear chromatin (**Butchkoet al., 2006**). This agree with (**Omar, 2009**) who showed presence of cytoplasmic vacuoles in pyramidal cells of the frontal cerebral cortex of rats following 8 weeks of ASPintake.

In the current study, the experimental group showed a splitting in some areas of the fiber myelin sheath.

This splitting resulted from the action of excitotoxins on multiple sites in the CNS that cause myelin stripping from fibers and damaging the neurons (**El–Sakhawy and Saeid, 2014**).

This in harmony with (**Abd El-Samad, 2010**) who showed that administration of Asp could cause splitting in myelin sheath fibers in the rat adult cerebellum.

Sobaniec et al., (2001)believed that the disruption of myelin or its loss occur as a result of changes in the basic protein of myelin, which can occur after disruption to the myelinating cell or as a result to axonal degeneration.

Lehning et al., (2002)recorded that the axonal degeneration in the cerebellum constitutes a component of a dying-back process of neuronal injury

These results are in harmony with (**Okasha, 2016)** who stated that ASP administration led to stripping of myelin sheath in the sciatic nerve.

In the present study, morphometric results of the diameter of granule cells nuclei at PND21 and PND60 in the experimental group showed highly significant decrease in comparison to age matching control group. **Mohamady and Mohamed, (2020)**showed that the granular layer had cells with highly dense nuclei separated by empty spaces between them after 6 weeks of Aspartame administration.

Conclusion

ASP could have a damaging effect on the structure of the cerebellum of the offspring of albino rats who born to mothers received ASP during pregnancy and lactation.

Abbreviations: Aspartame (ASP), postnatal day (PND),diketopiperazine (DKP),external granular

layer (EGL), internal granular layer (IGL),and molecular layer(ML)

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