Original Article	**A Study of The Effect of Prenatal Exposure to Valproic Acid on The Cerebellum of Albino Rat's Offspring and The Possible Protective Role of Folic Acid**
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ABSTRACT

Abstract: Valproic acid (VPA) is used as an anticonvulsant for the treatment of epilepsy. It is also used as one of the mood stabilizing agents in patients with anxiety disorder and as a prophylactic treatment of migraine. The need for anticonvulsant prophylaxis in women of childbearing period is essential since convulsive seizures are considered harmful to the developing embryo. Exposure to VPA during the first trimester of pregnancy was found to be associated with increased risks of several congenital malformations and also in utero exposure to VPA in rats caused cerebellar anomalies.

Folic acid (vitamin B9) is essential for the DNA synthesis and certain biological reactions. Adequate folate intake helped in protection against congenital malformations including neural tube defects.

Aim of the work: To investigate the effect of prenatal exposure to valproic acid on the cerebellum of albino rat's offspring and to clarify the possible protective role of folic acid.

Material and Methods: Twenty four pregnant albino rats were divided into four groups, six rats each. Group (A); a control group. Group (B); rats received sodium valproate (400 mg/Kg. B.W) starting on gestational day 13 daily till the end of pregnancy by oral gavage. Group (C); rats received folic acid (4 mg/Kg. B.W) starting from first day of pregnancy daily till the end of pregnancy by oral gavage. Group (D); rats received folic acid as in group (C) concomitantly with sodium valproate as in group (B) by oral gavage. At the end of the experiment, six male pups from each group were sacrificed on the postnatal day 14. The cerebella were extracted and processed for light microscopic examination using H&E for paraffin sections and toluidine blue for semi-thin sections. Also immuno-histochemistry technique for glial fibrillar acidic protein (GFAP) was applied to demonstrate astrocytes. Morphometric study was conducted to measure the thickness of cerebellar cortex, count the number of apparently normal Purkinje cells and number of Bergmann cells and calculate GFAP percentage area.

Results: The cerebellar cortex of the control group (A) consisted of three layers; the molecular layer, the Purkinje cell layer and the granular layer. The molecular layer appeared pale with few stellate cells and basket cells. The Purkinje cell layer consisted of Purkinje cells arranged in single row with their oval or flask shaped cell bodies, pale central nuclei and apical cytoplasmic cones. The granular layer consisted of deeply stained rounded small granule cells. This general architecture of the cerebellar cortex was greatly affected in group (B) and an external granular layer was detected on the cerebellar surface in some sections. The Purkinje cells appeared degenerated with pyknotic nuclei and the number of apparently normal cells was statistically decreased compared to control. The number of Bergmann cells was statistically significantly increased. Some granule cells were degenerated with pyknotic nuclei. Also astrocytes showed a strong positive reaction to GFAP with statistically significantly high percentage area compared to the control group. Examination of group (D) showed apparently normal arrangement of layers, Purkinje cells restored their normal flask shaped appearance and arranged in single row with Bergmann glia inbetween. Also astrocytes gave a mild positive reaction to GFAP similar to control group.

Conclusion: Valproic acid (VPA) had degenerative structural effect on the cerebellar cortex if administered during pregnancy. VPA should be administered at the least effective dose and that folic acid co-administration is highly recommended to reduce its toxic effect.

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INTRODUCTION:

The word cerebellum is the Latin diminutive form of cerebrum which means little brain. It is part of the brain that plays an important role in motor control and cognitive functions as language and attention and regulating fear and pleasure responses *(Wolf et al. 2009)*. In mice, the cerebellum was an ideal system for studying the effect of different substances on neuronal development as it is immature at birth and continues to develop in the first weeks of postnatal life *(Watson et al. 2006)*.

Epilepsy affects 1-2% of humans worldwide and has a peak incidence in the first year of life (Rogawski and Loscher, 2004). Valproic acid is a first-choice agent for most forms of epilepsy; also intravenous valproate is of value in the treatment of status epilepticus (Perucca, 2002). It is also used in treatment of bipolar mania and in migraine prophylaxis *(Kinze et al. 2001)*.

The need for anticonvulsant prophylaxis in women of childbearing potential couldn't be prevented since convulsive seizures are also considered harmful to the developing embryo *(Adab et al. 2001)*, so physician's approach involved choosing the anti - epileptic drug that is most likely to control the patient's seizure type(s) at relatively low dosages (Perucca, 2002).

Exposure to VPA during the first trimester of pregnancy was found to be associated with increased risks of congenital malformations, such as spina bifida, atrial septal defect, cleft palate and polydactyly *(Jentink et al. 2010)*. *Meador et al. (2006)* declared that children of mothers taking valproate during pregnancy are at risk for significantly lower IQs. Also in utero exposure of rats to valproic acid causes cerebellar anomalies *(Ingram et al. 2000)*.

Folic acid or folate (vitamin B9) is essential for numerous body functions. Humans cannot synthesize folate therefore folic acid has to be supplied through the diet to meet their daily requirements. The human body needs folate to synthesize DNA and to act as a cofactor in certain biological reactions, cell division and growth *(Pietrzik et al. 2010)*.

Adequate folate intake with pregnancy helps protect against a number of congenital malformations, including neural tube defects as spina bifida and anencephaly (Morrell, 2002)

Therefore, the present study was carried out to investigate the effect of prenatal exposure to valproic acid on cerebellum of albino rat's offspring and the possible protective role of folic acid.

MATERIAL AND METHODS

Drugs:

A. Sodium Valproate (oral solution) was purchased from Sanofi Company (Paris, France)

B. Folic Acid (tablets) was purchased from General Nutrition Corporation (Pittsburg, U.S.A)

Experimental design:

After obtaining the approval of the Committee of Animal Research Ethics (CARE), thirty adult female rats (weighing 180 - 200 gm) were locally bred at the animal house of Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. Each three rats were housed in a separate plastic cage in light controlled air-conditioned room (21-23 C°) under routine conditions with free access to food and water. Each two females at pro-estrous phase were caged with one male for a day and next day a vaginal smear was examined for sperms which indicated positive conception (day zero). Then twenty four pregnant rats were classified into four groups:

Group A: A control group of six pregnant rats which received basal standard diet and free water access.

Group B: Six pregnant rats received sodium valproate at a dose of 400 mg/Kg. B.W starting on gestational day 13 daily till the end of pregnancy by oral gavage *(Amel et al. 2012)*.

Group C: Six pregnant rats received folic acid dissolved in distilled water at a dose of 4 mg/

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kg. B.W. daily *(Dawson et al. 2006)* starting from first day of pregnancy till the end of pregnancy by oral gavage *(Aktas et al. 2010)*.

Group D: Six pregnant rats received folic acid as in group (C) concomitantly with sodium valproate as in group (B) by oral gavage.

At the end of the experiment the offspring of all groups were left housed with their mothers then six male pups from each group were sacrificed on the postnatal day fourteen (PD 14) by a lethal dose of anesthesia according to the protocol of the CARE of Ain Shams University. The heads were separated first then the cerebella were dissected out.

Each cerebellum was bisected into two halves; one half was immersed in Bouin's solution for 1-2 days and small pieces were processed to paraffin blocks. Sections 5-7 um were stained by haematoxylin and eosin (Bancroft and Gamble, 2013). Other paraffin sections were subjected to Glial fibrillary acidic protein (GFAP) immuno- histochemistry to examine the distribution of astrocytes and their response to neural degeneration or injury. Primary anti GFAP antibody Goat polyclonal IgG, anti-rat produced by Dako Cytomation was used. Working dilution was 1: 1000. Immuno-histochemical reaction was carried out using the avidin biotin peroxidase system followed by incubation with 1/100 normal rabbit serum for 20 minutes in order to omit non- specific background. GFAP containing cells (astrocytes) appeared brown (Chen and Weber, 2002).

The other half of cerebellum was fixed in 3% phosphate buffered glutaraldehyde and small pieces (1mm³) were processed to epon blocks. Semi-thin sections were cut and stained with 1% toluidine blue (Bancroft and Gamble, 2013). All sections were examined by light microscope.

Computer image analysis:

The TS View image analysis software in the Anatomy Department, Faculty of Medicine, Ain Shams University, was employed. The thickness of cerebellar cortex was measured in the H&E sections using digital photomicrographs taken at magnification of 100. The number of apparently normal Purkinje cells and the number of Bergmann glial cells were counted in H&E sections using digital photomicrographs taken at magnification of 1000. The GFAP percentage areas were measured in the GFAP immuno-histochemisrty stained sections using digital photomicrographs taken at magnification of 400. All sections were examined using an Olympus CX31 microscope equipped with digital camera. Six randomly chosen fields in six sections obtained from six different animals from the same group were used for measuring.

Statistical analysis: Statistical measures were done using the statistical package for social studies (SPSS – Version 13.0). One way analysis of variance (ANOVA) was employed to compare means between groups. Bonferroni Post hoc test was used to detect significance between every two individual groups.

The significance of the data was determined by the probability *(P-value)*. *P>0.05* was considered insignificant, *P≤0.05* was considered significant and *P≤0.01* was considered highly significant. Data were represented in tables and column charts.

RESULTS

Histological results

Group A:

Examination of haematoxylin and eosin and toluidine blue stained sections of this group showed developed cerebellar cortex with large folia and deep fissures and consisted of three layers; the molecular layer, the Purkinje cell layer and the granular layer (Fig. 1).

The molecular layer appeared pale with few stellate cells and basket cells (Fig. 2). The Purkinje cell layer consisted of Purkinje cells arranged in single row with their oval or flask shaped cell bodies, pale central nuclei (Figs. 2, 3&4) and apical cytoplasmic cones (Fig. 3). Bergmann cells with their pale nuclei appeared between Purkinje cells (Figs. 2, 3&4).

The granular layer was formed of deeply stained rounded small granule cells and cerebellar islands formed of numerous nerve synapses inbetween (Figs. 2, 3&4).

Examination of the GFAP immuno-histochemistry stained sections of this group showed mild positive reaction for astrocytes in all cortical layers (Fig. 5)

Group (B):

Examination of haematoxylin and eosin and toluidine blue stained sections showed cerebellar cortex with large folia and deep fissures inbetween (Figs. 6&7). Some sections showed cerebellar cortex consisted of the molecular layer, Purkinje

cell layer and granular layer like the control group (Fig. 6). In some sections the cerebellar cortex showed an external granular layer, which was absent in the control group, the molecular layer with Purkinje cell layer and an internal granular layer (Fig. 7).

The molecular layer showed areas of degeneration and most of the Purkinje cells were shrunken and degenerated with pyknotic nuclei and areas of cell loss were detected (Figs. 8, 9&10). The granular layer showed some degenerated granule cells (Fig. 9). In addition to degenerated Purkinje cells with pyknotic nuclei, other necrotic distorted shaped cells with pale nuclei were seen with abundant Bergmann glial cells (Figs. 11&12).

In GFAP immunohistochemistry stained sections, a strong positive reaction was detected in all cortical layers (Fig. 13).

Group (C):

Examination of haematoxylin and eosin and toluidine blue stained sections of this group showed similar findings as those of group (A). The cortex was made up of folia with fissures inbetween, the three layers of cortex were distinguishable; the molecular, the Purkinje cell layer and the granular layer (Fig.14). The characteristic flask shaped Purkinje cells with their central nuclei and cytoplasmic cones were arranged in a single row having Bergmann glial cells scattered inbetween (Figs. 15, 16&17). Small rounded granule cells were detected in the granular layer (Fig. 15). A mild positive reaction was detected in GFAP immuno-histochemistry stained sections (Fig. 18).

Group (D):

Examination of haematoxylin and eosin and toluidine blue stained sections of this group showed The cortex was made up of folia with fissures inbetween, distinguishable layers of cerebellar cortex; the molecular layer, Purkinje cell layer and granular layer (Fig. 19). Most of the Purkinje cell layer consisted of apparently normal flask shaped Purkinje cells arranged in one row with pale nuclei and apical cytoplasmic cones while few cells appeared distorted in shape with deeply stained nuclei (Figs. 20&21). Bergmann cells were detected between the Purkinje cells.

Granular cells appeared with deeply stained nuclei with cerebellar islands in between (Fig. 22).

The GFAP Immuno-histochemistry stained sections showed mild positive reaction in all cortical layers if compared to group (B). Astrocytes appeared as star shaped cells within the granular layer and with their processes extending in all cortical layers (Fig. 23).

Morphometric results

Thickness of cerebellar cortex:

There was a statistical significant decrease in the thickness of cortex in group B in comparison with group A (control) with p-value 0.0493 and a non-significant decrease in the thickness of cortex of group C in comparison with group A with p-value 0.8801, and a non-significant decrease between groups D and A with p-value 0.5414 as seen in table (1) and chart (1).

Number of apparently normal Purkinje cells/ HPF:

There was a highly statistical significant decrease in number of apparently normal Purkinje cells between group B and group A (control) with p-value 0.0092 and a non-significant decrease between group C and A with p value 0.6821. There was a non-significant decrease between groups D and A with p value 0.2665 as shown in table (2) and chart (2).

Number of Bergmann glial cells/ HPF:

There was a highly statistical significant increase in number of Bergmann glial cells in group B in comparison to A (control) with p-value 0.0007 and a non-significant increase between group C and A with p-value 0.834. A highly statistical significant decrease in number of cells was detected in group D when compared to group B with p-value 0.0001 as shown in table (3) and chart (3).

The GFAP percentage area:

There was a highly statistical significant increase in GFAP percentage area in group B when compared to A (control) with p-value 0.00001, and a non-significant increase between groups C and A with p-value 0.891. A highly significant decrease was detected in group D when compared to group B with p-value 0.00001 as shown in table (4) and chart (4).
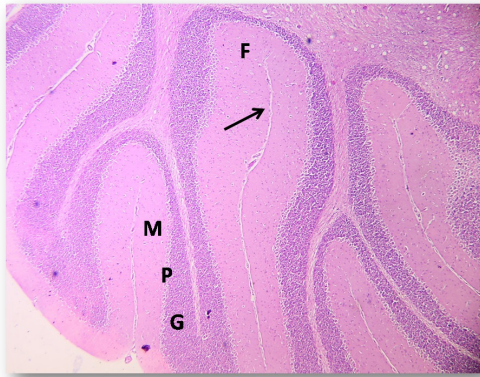
Fig. 1: A photomicrograph of a section in the cerebellum of a rat of group (A), showing prominent cerebellar folia (F) with deeper fissures (arrow) and well developed cortex buildup of three layers; molecular layer (M), Purkinje cell layer (P) and granular layer (G). (H&E x 100)
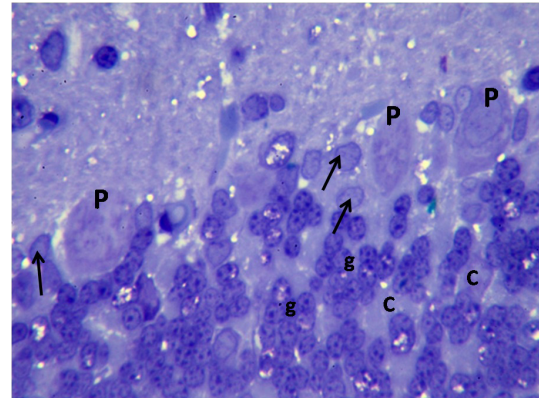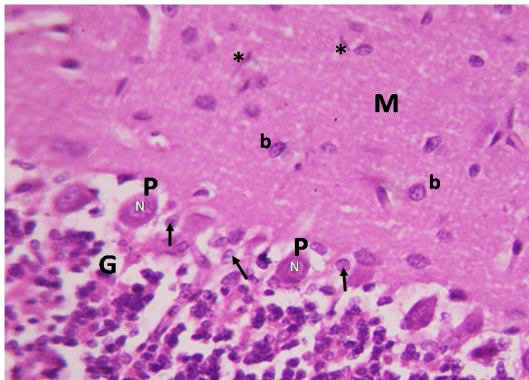

Fig. 2: A photomicrograph of a section in the cerebellum of a rat of group (A), showing cerebellar cortex buildup of pale molecular layer (M) containing stellate cells (*) and basket cells (b). The Purkinje cell layer (P) containing oval shaped Purkinje cells with their central nuclei (N) that are surrounded by Bergmann cells (arrow). Note the granular layer (G) with small rounded deeply stained granule cells. (H&E x 400)
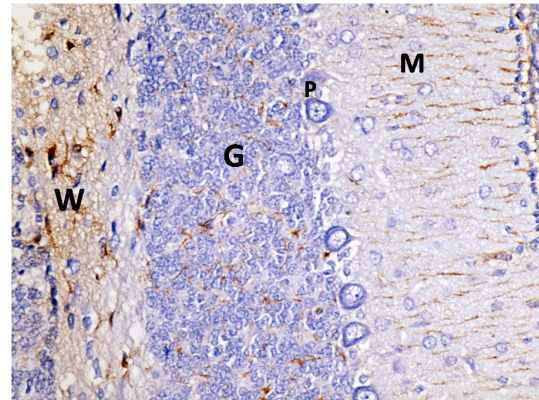

Fig. 3: A photomicrograph of a section in the cerebellum of a rat of group (A), showing the flask shaped Purkinje cells (P) with apical cytoplasmic cones (black arrows) and pale central nuclei (N). Bergmann glial cells (red arrow) appear between the Purkinje cells. Note the closely packed small granule cells (g) and the clear areas of cerebellar islands (C) inbetween.

(H&E x1000)

Fig. 4: A photomicrograph of a semi-thin section in the cerebellum of a rat of group (A), showing flask shaped Purkinje cells (P) with large pale nuclei arranged in a single row and Bergmann cells with pale nuclei (arrow). Small rounded deeply stained granule cells (g) with clear cerebellar islands (C) inbetween are observed. (Toluidine blue x 1000)

Fig. 5: A photomicrograph of a section in the cerebellum of a rat of group (A), showing a mild positive reaction of GFAP immuno-histochemistry for astrocytes in all cortical layers; molecular layer (M) with Purkinje cell layer (P) , granular layer (G) and white matter (W). (GFAP x 400)
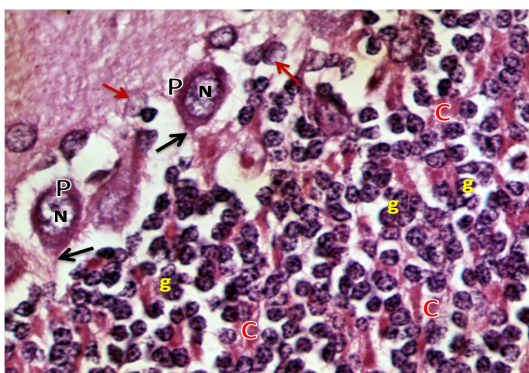

Fig. 6: A photomicrograph of a section in the cerebellum of a rat of group (B), showing cerebellar folia (F) with deep fissures(*). The cortex consists of molecular layer (M), Purkinje cell layer (P) and granular layer (G). (H&E x 100)
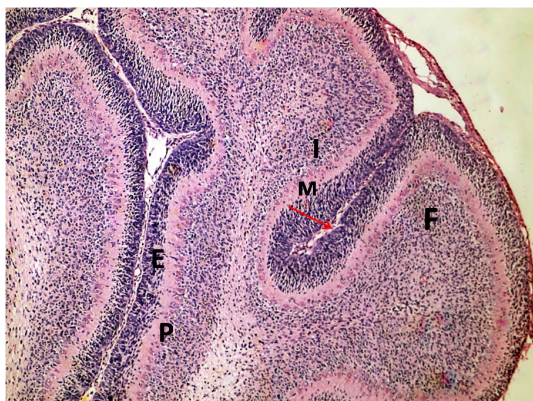
Fig. 7: A photomicrograph of a section in the cerebellum of a rat of group (B), showing folia (F) and fissures (arrow). The cortex consists of external granular layer (E), molecular layer (M) with Purkinje cell layer (P) and internal granular layer (I).
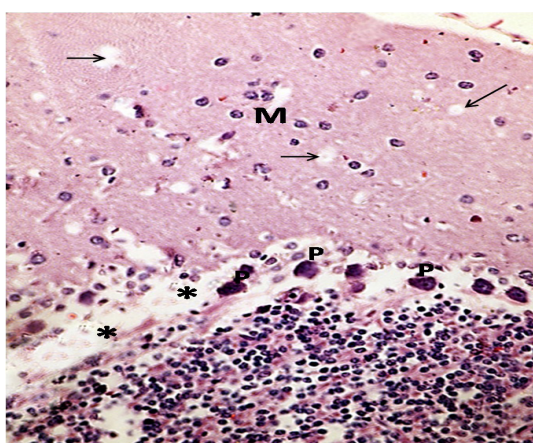
(H&E x 100)

Fig. 8: A photomicrograph of a section in the cerebellum of a rat of group (B), showing the molecular layer (M) with areas of degeneration (arrow). The Purkinje cell layer shows shrunken and degenerated Purkinje cells (P) with pyknotic nuclei and areas of Purkinje cell loss are obvious (*). (H&E x 400)
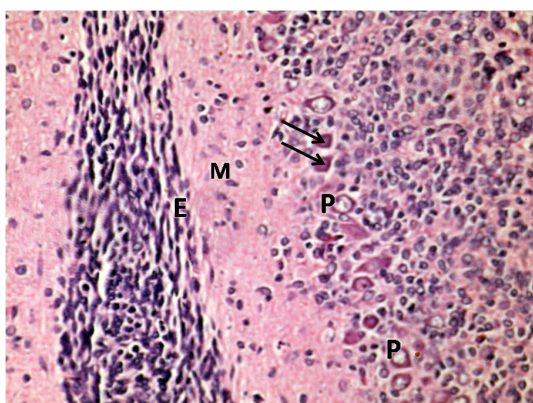

Fig. 9: A photomicrograph of a section in the cerebellum of a rat of group (B), showing external granular layer (E), molecular layer (M) with Purkinje cell layer (P). Some Purkinje cells appear shrunken and degenerated with pyknotic nuclei (arrow). (H&E x 400)
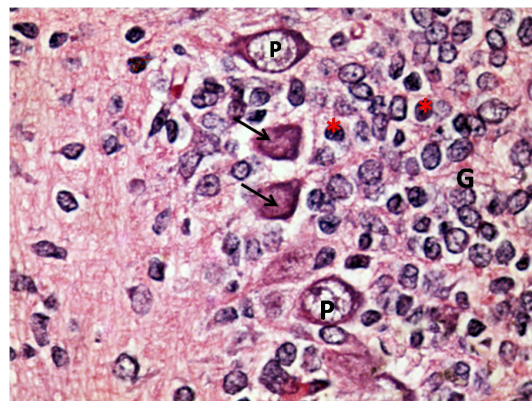

Fig. 10: Higher magnification of previous photomicrograph showing some degenerated Purkinje cells with pyknotic nuclei (arrow), others appear normal (P). The granular layer (G) shows some degenerated granule cells (*). (H&E x 1000)
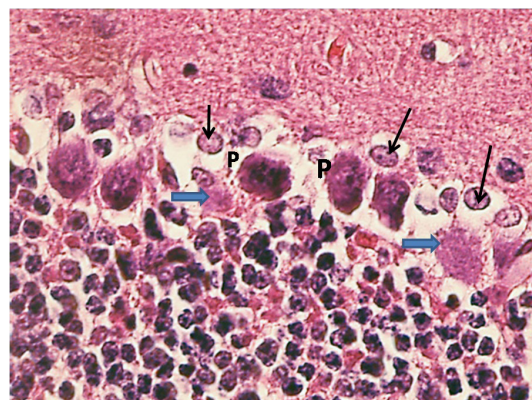

Fig. 11: A photomicrograph of a section in the cerebellum of a rat of group (B), showing dark degenerated Purkinje cells (P) and other pale distorted shaped necrotic cells (blue arrow). Many Bergmann glial cells (black arrow) are present between the Purkinje cells. (H&E x 1000)
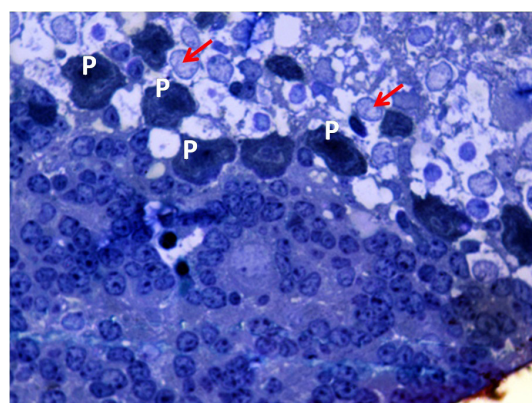

Fig. 12: A photomicrograph of a semi-thin section of a rat of group (B) showing many degenerated Purkinje cells (P) surrounded by many Bergmann glial cells (arrow).
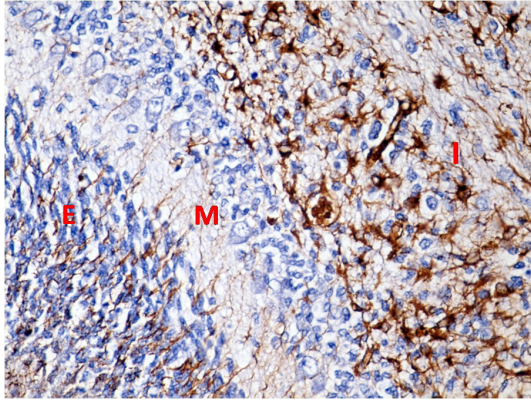
(Toluidine Blue x 1000)

Fig. 13: A photomicrograph of a section in the cerebellum of a rat of group (B), showing a strong positive reaction of GFAP immuno-histochemistry for astrocytes cell bodies and dendrites in all layers; the external granular (E), molecular (M) and the internal granular layer (I).   (GFAP x 400)
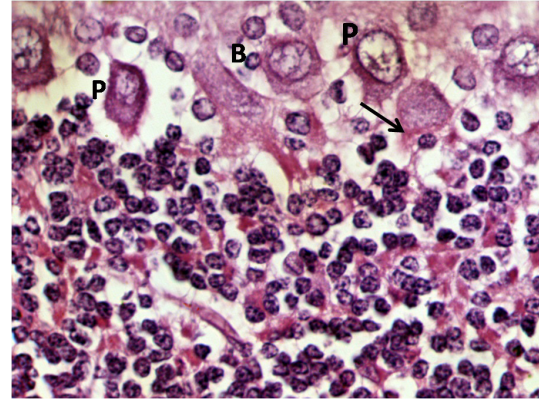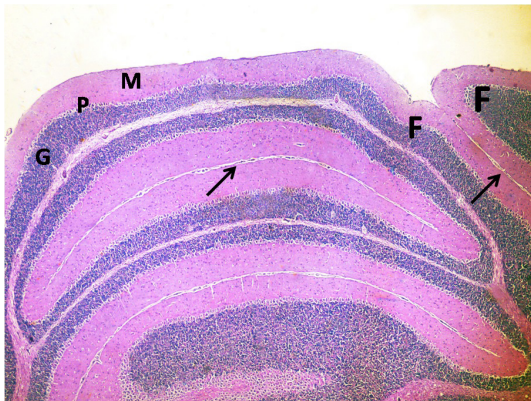

Fig. 14: A photomicrograph of a section in the cerebellum of a rat of group (C), showing the cerebellar folia (F) with fissures (arrow) inbetween. The cortical layers consist of molecular layer (M), Purkinje cell layer (P) and granular layer (G).

(H&E x 100)

Fig. 15: A photomicrograph of a section in the cerebellum of a rat of group (C), showing the cortical layers consisting of pale molecular layer (M), Purkinje cell layer (P) containing the flask shaped Purkinje cells with their pale nuclei (N) arranged in single row with their cytoplasmic cones (arrow) and granular layer (G) with packed small rounded cells.   (H&E x 400)

Fig. 16: A photomicrograph of a section in the cerebellum of a rat of group (C), showing the Purkinje cells (P) with their pale nuclei arranged in one row and apical cytoplasmic cone (arrow) with Bergmann glial cells inbetween (B).
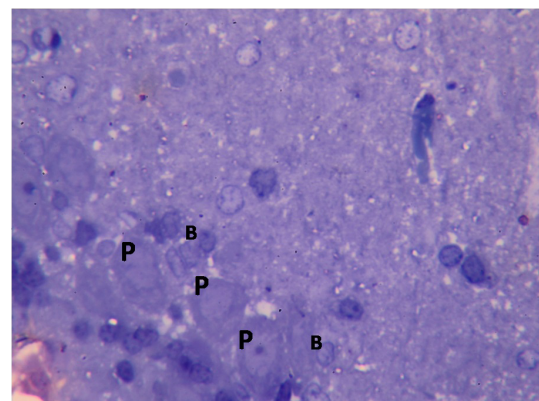
(H&E x 1000)

Fig. 17: A photomicrograph of a semi-thin section in the cerebellum of a rat of group (C), showing the Purkinje cells (P) with their pale nuclei arranged in single row with Bergmann cells (B) inbetween.   (Toluidine Blue x 1000)

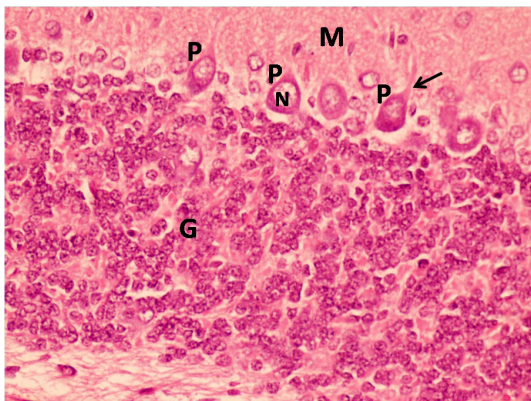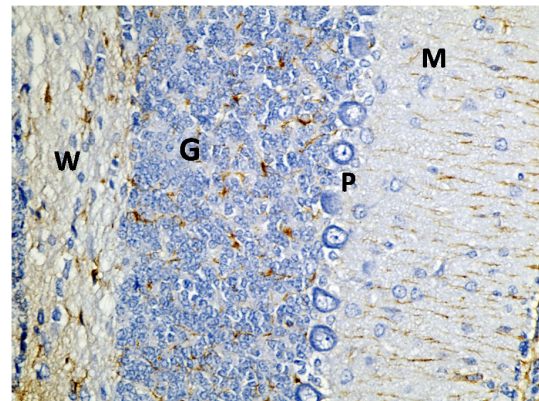**Fig. 18:** a photomicrograph of a section of a rat of group (C) showing mild positive reaction of GFAP immuno-histochemistry for astrocytes in the molecular layer (M), Purkinje cell layer (P), granular layer (G) and white matter (W).

(GFAP x 400).

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Fig. 19: A photomicrograph of a section in the cerebellum of a rat of group (D), showing the cerebellar folia (F) with fissures (arrow) inbetween. The cortical layers consisting of molecular layer (M), Purkinje cell layer (P) and granular layer (G).
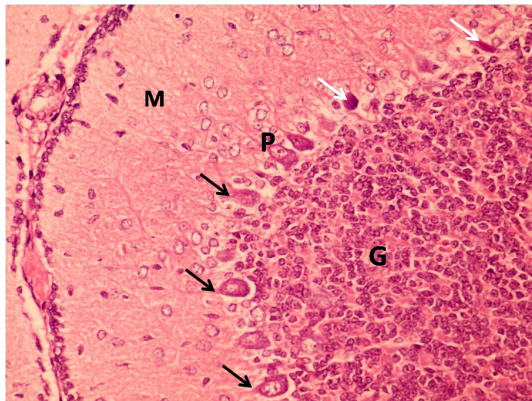
(H&E x 100)

Fig. 20: A photomicrograph of a section in the cerebellum of a rat of group (D), showing molecular layer (M), Purkinje cell layer (P) with some apparently normal Purkinje cells with pale nuclei and apical cytoplasmic cone (black arrows), others appear degenerated with pyknotic nuclei (white arrows) and granular layer (G) with deeply stained granule cells.
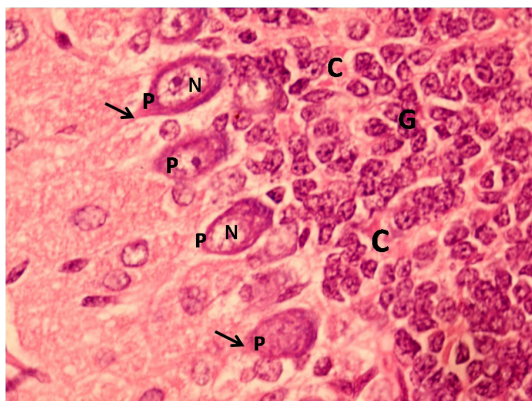
(H&E x 400)

Fig. 21: A photomicrograph of a section in the cerebellum of a rat of group (D), showing Purkinje cell layer with apparently normal Purkinje cells (P) with pale nuclei (N) and apical cytoplasmic cones (arrows), granular layer (G) with deeply stained small granule cells and cerebellar islands inbetween (C).

(H&E x 1000)
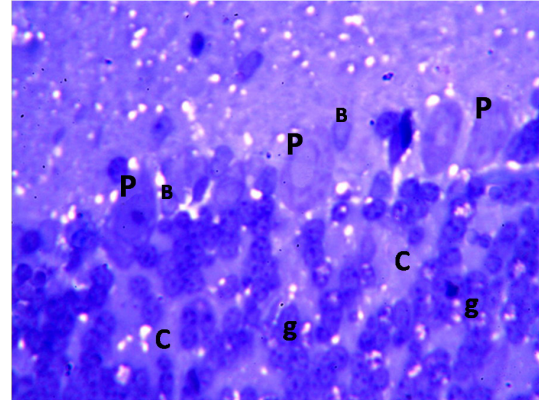
Fig. 22: A photomicrograph of a semi-thin section of a rat of group (D) showing flask shaped Purkinje cells (P) arranged in one row with pale nuclei. Bergmann glial cells (B) are seen between the Purkinje cells. Granular layer with small dark granule cells (g) and cerebellar islands (C) are obvious.

(Toluidine blue x1000).
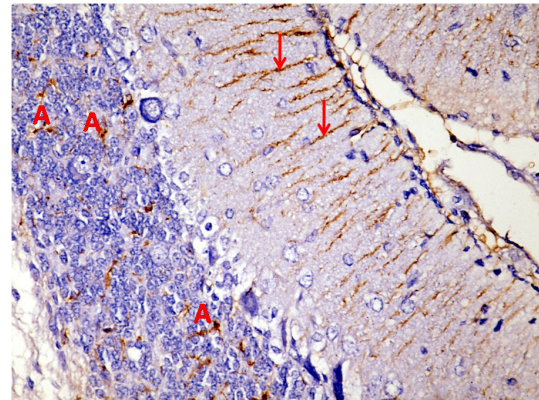
Fig. 23: A photomicrograph of a section in the cerebellum of rat of group (D), showing a mild positive reaction of GFAP immuno-histochemistry for astrocytes cell bodies (A) and dendrites (arrow) in all layers.   (GFAP x 400)

Table (1): Means ± SD of the thickness of the cerebellar cortex in um/ LPF in all groups at the age of postnatal day 14.

Group (day fourteen)	A (control)	B (VPA)	C (F.A)	D (VPA + F.A)
Thickness of cortex (um)	398.779 ± 169.879 um	243.524 ± 7.974 um	384.331 ± 153.401 um	345.199 ± 119.292 um
p-value		0.0493 ᵃ	0.8801	0.5414

a) Significant decrease than control.

Chart (1): Means of thickness of cerebellar cortex in um /LPF in all groups at the age of postnatal day 14.

Table (2): Means ± SD of number of apparently normal Purkinje cells / HPF in all groups at the age of postnatal day 14.

Group (day fourteen)	A (control)	B (VPA)	C (F.A)	D (VPA + F.A)
Number	4.833 ± 1.471	2.333 ± 1.21	4.333 ± 2.503	3.833 ± 1.471
p-value		0.0092 [a]	.6821	0.2665

a) Highly significant decrease than the control

Chart (2): Means of number of apparently normal Purkinje cells/HPF in all groups at the age of postnatal day 14.
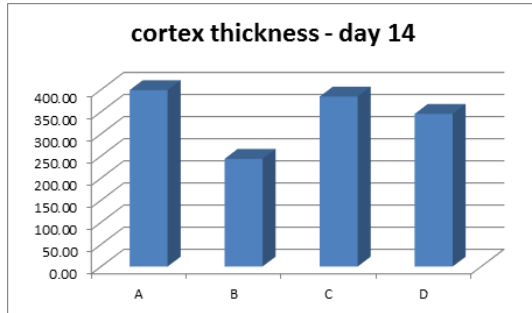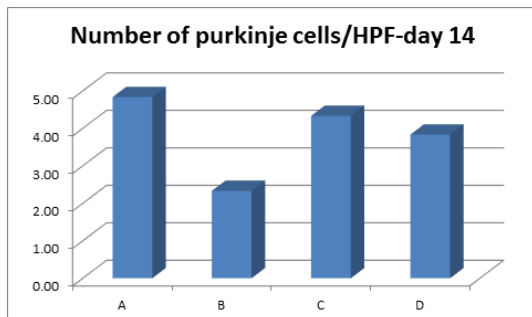
Table (3): Means ± SD of number of Bergmann glial cells/HPF in all groups at the age of postnatal day 14.

Group (day fourteen)	A (control)	B (VPA)	C (F.A)	D (VPA + F.A)
Number	8.333 ± 3.265	16 ± 2.804	8.751 ± 3.429	8.333 ± 1.861
p-value		0.0007 [a]	0.834	0.0001 [b]

a) Highly significant increase than control

b) Highly significant decrease than group B
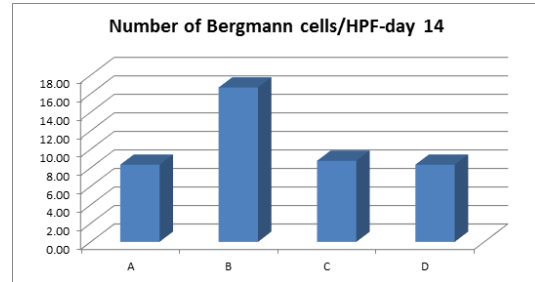
Chart (3): Means of number of Bergmann glial cells/HPF in all groups at the age of postnatal day 14.

Table (4): Means ± SD of GFAP area percentage/HPF in all groups at the age of postnatal day 14

Group (day fourteen)	A (control)	B (VPA)	C (F.A)	D (VPA + F.A)
GFAP Area percentage	2.258 ± 2.768	25.231 ± 6.401	2.291 ± 2.562	3.389 ± 2.292
p-value		0.00001 [a]	0.891	0.00001 [b]

a) Highly significant increase than control

b) Highly significant decrease than group B
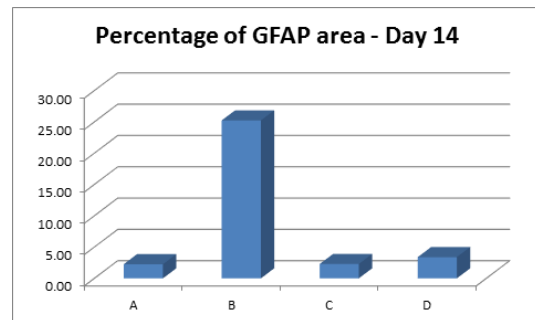
Chart (4): Means of GFAP area percentage/HPF in all groups at the age of postnatal day 14.

DISCUSSION

Valproic acid (VPA) is used as an anticonvulsant and mood-stabilizing agent for the treatment of epilepsy and bipolar disorder (Bowden and Singh, 2005) and as a prophylactic treatment of migraine *(Mathew at al., 2000)*.

Valproic acid is generally regarded as a first-choice agent for most forms of idiopathic and symptomatic generalized epilepsies including tonic-clonic, myoclonic and absence seizures, also administration of intravenous valproate could be of value in the treatment of status epilepticus (Perucca, 2002).

According to the FDA valproic acid is a pregnancy category D drug when used in the management of epilepsy, and manic episodes associated with bipolar disorder and should be only used when the physicians decide that the benefits outweighed the potential risks or other drugs failed to control the seizures since convulsive seizures are also considered harmful to the developing embryo (Perucca, 2002).

Exposure to VPA during the first trimester of pregnancy was found to be associated with increased risks of congenital malformations, such as spina bifida, atrial septal defect, cleft palate and polydactyly *(Jentink et al. 2010)*. Another study showed children of mothers taking valproate during pregnancy are at risk for significantly lower IQs *(Meador at al., 2006)*. Also in utero exposure of rats to valproic acid causes cerebellar anomalies *(Ingram et al. 2000)*.

Volpe (2009) stated that in humans, a rapid growth in cerebellum development takes place in the third trimester. This is in contrast to the development of the cerebellum in the commonly used animal model system, the rodent, in which the cerebellum is relatively immature at birth, and the proliferation of the external granular layer, the formation of the internal granular layer occur postnatal *(Correlas et al. 2006)*. Therefore, the rat was the animal of choice in the current study as the neurodevelopment in the early postnatal days in rat is correspondent to the human development in the mid to late gestational periods.

In rats, postnatal day 14, corresponds to the third trimester of human development when cerebellar and hippocampal granule cells migrate and differentiate, therefore it is considered a critical period in neural development (Rice and Barone Jr, 2000).

Many studies have evaluated the time period when exposure to VPA would cause the greatest effect, *Kim et al. (2011)* examined the critical period necessary to induce autism-like behavior in rats using prenatal VPA exposure. Pregnant rats received injections of 400 mg/kg of VPA at gestational days (GDs) 7, 9.5, 12 and 15. The results of the sociability experiment showed that rats exposed to VPA at GD 12 were less sociable than both the control group and animals exposed at GDs 7, 9.5, and 15. Also the cerebellar affection in many experiments was recorded on administration of valproic acid on gestational day 12.5 *(Schneider et al. 2006 and Dendrinos et al. 2011)* as well as on gestational day 13 *(Gandal et al. 2010)*.

In the current study the examination of cerebellar cortex of offspring born to mothers treated with valproic acid (group B) showed the presence of external granular layer on the cerebellar cortical surface if compared to control group which may be an indicator of delayed maturation process that was in agreement with *Fukuda et al. (2005)* who suggested that depression of the synthesis of neurotrophins such as BDNF (brain derived neurotrophic factor) and NT-3 (Neurtrohin 3) which prevent the neurons from initiating programmed cell death and induce differentiation of progintor cells to form neurons and reach maturation might be the cause of delayed maturation of cerebellar cortical layers.

The appearance of areas of degeneration in the molecular layer and between the Purkinje cells was mostly due to shrinkage of Purkinje cells and the withdrawal of their protoplasmic processes secondary to degeneration of their cytoskeletal elements as mentioned by (Sobaniec-Lotowska, 2001)

In the present study, the Purkinje cells also appeared shrunken, distorted and deeply stained. In agreement to these findings, *Troudi et al. (2012)* and shalaby and Sarhan (2008) stated that long term valproic acid administration caused considerable damage to structural and functional biosynthesis of cell proteins manifested markedly by increased electron density.

Other authors believed that pyknotic nuclei appearance was due to ischemia as a result of affection of capillary wall of cerebellar cortex with subsequential disorders in the transportation of valproic acid or its toxic metabolites through the structural elements of blood brain barrier to neurons (Sobaniec-Lotowska and Lotowska, 2005).

Other studies suggested that these pyknotic nuclei might be a certain phase of apoptosis as these cells displayed markedly condensed cytoplasm and nucleoplasm (Sobaniec-Lotowska, 2001).

Yamada and Watanabe (2002) concluded the correlation of the Bergmann neuroglial cells in the process of the migration, dendritogenesis, synaptogenesis and maturation of Purkinje cells. Similarly, *Wang et al. (2012)* reported that Bergmann glia play a precious role in controlling the membrane potential and thereby the activity of the adult Purkinje cells.

In the current work the morphometric study revealed that the number of Bergmann glial cells was significantly increased in sodium valproate treated group with a significant decrease in the

thickness of cerebellar cortex of group B. Naruse et al. (2013) described the Bregmann glia as a precursor for the astrocytes. These findings could explain why the GFAP immuno-histochemistry in the present study showed strong positive reaction if compared to control and it was confirmed by morphometric study showing a statistically significant increase of its area percentage in group B. GFAP is an intermediate filament protein that is expressed by numerous cell types of the CNS including astrocytes and ependymal cells. GFAP is considered to be important in modulating astrocyte motility and shape by providing structural stability to astrocytic processes and also as a specific marker of mature astrocytes of the central nervous system *(Eng et al. 2000)*.

In the CNS, following injury, either as a result of trauma, disease, genetic disorders, or chemical insult, astrocytes become reactive and respond in a typical manner, termed astrogliosis. Astrogliosis is characterized by the rapid synthesis of GFAP and is shown by an increase in protein content or by immunostaining with the GFAP antibody. So the gliosis could be a result of toxic effect of VPA on neurons that was in agreement with results of *ElSawy et al. (2013)*.

The granular cells also appeared degenerated with pyknotic deeply stained nuclei which could be explained by neuroapoptosis which is caused by the VPA (Sobaniec-Lotowska, 2001).

The results of group D showed a remarkable improvement in the cerebellar cortical structure with folic acid administration, with distinguishable cortical layers, Purkinje cells were appearantly normal with their oval or flask shaped apparance and pale nuclei and arranged in single row with few distorted Purkinje cells. Also the number of Bergmann cells was statistically of no significant difference compared to control.

Folate acts as a cofactor for enzymes involved in DNA and RNA biosynthesis. Folate is also involved in the supply of methyl groups to the so-called methylation cycle, which uses methionine from homocysteine, an amino acid that is related to many neurodegenerative diseases. A high level of this amino acid can lead to brain damage and cognitive disturbances. The synthesis of methionine thus prevents the accumulation of this harmful amino acid in the brain (Viatcheslav Wlassoff 2014). Methionine synthase plays another role as it converts circulating N5-methyltetrahydrofolate into tetrahydrofolate. The latter but not the former can act as a substrate

for polyglutamate synthase, thereby becoming retained in the cell as polyglutamate. Interruption of DNA biosynthesis or methylation reactions could prevent the proper closure of the neural tube. Such inhibition could be caused by simple deficiency of either folic acid or vitamin B12 *(Scott et. al. 1994)*

The GFAP percentage area was highly significantly decreased between groups D and B which indicated the decrease in astrogliosis and immunohistochemical reaction which was due to less toxic effect on the cerebellar cortex. These findings were in agreement with *Dawson et al. (2006) and Aktas et al. (2010)* who stated that folic acid helped in the protection against the toxic effect of VPA. Also *Dawson at al. (2006)* stated that folic acid protection might be mediated by prevention of VPA-induced alterations in proteins involved in neurulation or prevent VPA-induced oxidative stress.

Finally from the present work it is concluded that VPA had a toxic effect on the cerebellar cortex and the neuronal development and that if VPA should be administered with pregnancy it is recommended to be in the least effective doses and that folic acid administration is highly recommended to reduce the effects on the cerebellum of the offspring.

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دراسة تأثير التعرض لحمض الفالبرويك قبل الولادة علي مخيخ ذرية الفئران البيضاء و الدور الوقائي المحتمل لحمض الفوليك

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ملخص البحث

المقدمة : يستخدم عقار حمض الفالبرويك لعلاج الصرع والاضطراب الوجداني و السيطرة علي اضطرابات الحالة المزاجية في المرضي المصابون بالقلق و أيضا في الوقاية من نوبات الصداع النصفي. هناك حاجة إلى الوقاية من نوبات الصرع لدى النساء اللاتي لديهن القدرة على الإنجاب وذلك لأن نوبات الصرع تعتبر أيضا ضارة لنمو الجنين. وقد تبين أن التعرض لحمض الفالبرويك خلال الأشهر الثلاثة الأولى من الحمل يكون مرتبطا مع زيادة مخاطر العديد من التشوهات الخلقية وأيضا فإن التعرض الرحمي لحمض الفالبرويك في الفئران قد تسبب في تشوهات في تكوين المخيخ. حمض الفوليك) احد مكونات فيتامين ب (هو ضروري لتخليق الحمض النووي وبعض التفاعلات البيولوجية ويساعد تناول حمض الفوليك بكميات مناسبة في الوقاية من التشوهات الخلقية بما في ذلك عيوب الأنبوب العصبي.

الهدف من العمل: التحقق من تأثير التعرض قبل الولادة لحمض الفالبرويك على مخيخ ذرية الفئران البيضاء وتوضيح الدور الوقائي المفترض لحمض الفوليك.

الطريقة البحث: تم تقسيم أربع وعشرون أنثى حاملا من الفئران البيضاء إلى اربع مجموعات، ست فئران لكل مجموعة : المجموعة الأولى: المجموعة الضابطة. المجموعة الثانية (ب) تلقت الفئران لجرعة 400 مجم/كج من فالبروات الصوديوم اعتبارا من اليوم الثالث عشر من الحمل حتى نهاية الحمل بواسطة أنبوب تغذية عن طريق الفم. المجموعة (ج) تلقت الفئران جرعة 4 مجم/كج من حمض الفوليك اعتبارا من اليوم الأول من الحمل حتى نهاية الحمل بواسطة أنبوب تغذية عن طريق الفم. المجموعة (د): تلقت الفئران لحمض الفوليك كما في المجموعة (ج) بالتزامن مع فالبروات الصوديوم كما في المجموعة (ب) بواسطة أنبوب تغذية عن طريق الفم .في نهاية الدراسة التجريبية، تم التضحية بستة من الجراء الذكور من كل مجموعة في اليوم الرابع عشر بعد الولادة. تم استئصال المخيخ و تهيئته للفحص بالمجهر الضوئي بإستخدام صبغة الهيماتوكسيلين والايوسين لشرائح البرافين وصبغة التولدين الأزرق للشرائح شبه الرقيقة واستخدام الصبغة المناعية GFAP لإظهار الخلايا النجمية. تم استخدام الطريقة المورفومترية لقياس سمك قشرة المخيخ و عدد الخلايا الهرمية والتى تبدو طبيعية و خلايا برجمان و النسبة المئوية للمساحة المصبوغة بال GFAP في كل مجموعة.

النتائج: أظهرت المجموعة الضابطة أن قشرة المخيخ تتكون من ثلاث طبقات، طبقة من الخلايا الجزيئية و طبقة من الخلايا الهرمية و طبقة من الخلايا الحبيبية. ظهرت الطبقة الجزيئية شاحبة مع عدد قليل من الخلايا النجمية وخلايا السلال في حين تتكون طبقة الخلايا الهرمية من خلايا مرتبة في صف واحد ذوات اشكال مخروطية، ذات انوية مركزية شاحبة و مخاريط سيتوبلازمية طرفية. تتألف الطبقة الحبيبية من خلايا حبيبية صغيرة مستديرة ذات صبغة داكنة. هذا التنسيق العام لقشرة المخيخ تأثر بشكل كبير في المجموعة (ب) حيث تم الكشف عن الطبقة الحبيبية الخارجية على سطح المخيخ في بعض القطاعات إذا ما قورنت مع المجموعة الضابطة وكذلك ظهرت الخلايا الهرمية مع انوية ضامرة. اظهرت خلايا برجمان تزايدا إحصائيا بشكل ملحوظ. كما لوحظ تلف بعض الخلايا الحبيبية مع وجود انوية ضامرة بها. كما وتم الكشف عن تفاعل إيجابي قوي للمناطق المصبوغة ب GFAP مع نسبة عالية إحصائيا مقارنة مع المجموعة الضابطة. أظهر فحص المجموعة (د) ترتيب طبيعي للطبقات، واستعادة الخلايا الهرمية مظهرها العادي المخروطي وترتيبها في صف واحد مع خلايا برجمان. كما أعطت الخلايا النجمية رد فعل إيجابي خفيف إلى GFAP مماثلة للمجموعة الضابطة.

الخلاصة: التعرض قبل الولادة لحمض الفالبرويك كان له تأثير مدمر على التركيبة الهيكلية لقشرة المخيخ إذا اعطي خلال فترة الحمل. وينبغي أن يعطى حمض الفالبرويك في أقل جرعة مؤثرة، وينصح بشدة أن يعطى حمض الفوليك في نفس التوقيت للحد من تأثيره السام.