ORIGINAL ARTICLE

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Possible ameliorative effect of Vitamin C on cerebellar toxicity induced by gibberellic acid during late pregnancy and early postnatal periods in albino rats

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SUMMARY

Gibberellic acid (GA3) is a plant growth regulator, widely used in agriculture in Egypt. The goal of this study was to illustrate the histopathological effects of (GA3) on the growing cerebellar cortex and the possible ameliorative effect of vitamin C. Fifty female Sprague-Dawly rats were classified into the following groups: Group I (control group); Group II (GA3-treated group), which received intra-gastric daily dose of GA3 55 mg/kg from the 14th day of pregnancy until the day 14 after delivery; Group III (GA3 & Vitamin C treated group), which received intra-gastric daily dose GA3, 55 mg/kg simultaneously with 100 mg of Vitamin C /kg from the 14th day of pregnancy till day 14 after delivery; and Group IV (Vitamin C-treated group), which received intra-gastric daily dose 100 mg of Vitamin C / kg from the 14th day of pregnancy till day 14 after delivery. One month after delivery, cerebella of pups from all groups were extracted and examined. The cerebellar cortex of GA3-treated group revealed degenerated and displaced Purkinje and granular nerve cells with prominent spongiosis in the molecular layer. Vitamin C administration resulted in marked regression of the previously mentioned neurotoxic effects. In conclusion: results of the current study revealed that maternal exposure to GA3 during pregnancy and lactation caused delayed development of the offsprings' cerebellar cortex. The co-administration of Vitamin C greatly reduced these neuro-toxic effects of GA3 exposure.

Key words: Growing cerebellar cortex – Gibberellic acid – Histopathology – Vitamin C

Abbreviations:

ANOVA, Analysis of variance; DNA, deoxyribonucleic acid; EGL, external granular layer; GA3, gibberellic acid; GCL, granular cell layer; MCL, molecular cell layer; MSG, monosodium glutamate; PCL, Purkinje cell layer; PGRs, plant growth regulator hormones; ROS, reactive oxygen species

INTRODUCTION

Gibberellic acid (GA3) is one of the plant growth regulator hormones (PGRs) that are broadly utilized in agriculture in numerous countries including Egypt (Muthu et al., 2011). It plays a significant role in plant cellular processes through induction of cell division. GA3 is sustained in the bioactive form in soil for months. Individuals may be vulnerable to GA3 components in diet containing various sorts of fruits and vegetables treated with GA3, as well as through drinking water contaminated by GA3 (Salih et al., 2014).

Many studies have reported that the central nervous system is the main target organ for the chemicals PGRs (Cokugras and Bodur, 2003).The embryogenesis of the mammalian cerebellum is an

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amazing histological event during the central nervous system development (Tam et al., 2009). The cerebellum is a part of the brain that develops early during embryonic life. However, it gains its mature features few months postnatal (Christensson et al., 2007). Despite the consumption of a high oxygen rate, its defense mechanisms are low against oxidant damage, so it is vulnerable to oxidative stress and toxicity. (Cokugras and Bodur, 2003). Therefore, the cerebellum is more susceptible to developmental distortions (Christensson and Garwicz, 2005).

It has been shown that the toxicity of numerous xenobiotics, including PGRs, is accompanied by the formation of reactive oxygen species (ROS), provoking oxidative stress which interacts with tissues, leading to numerous pathophysiological changes and cell damage in many organs (Chaari-Rkhis et al., 2006; Ali et al., 2004)

Antioxidants are substances that may defend the body cells against the impacts of free radicals provoked by oxidative damage in different cellular compartments and tissues. Vitamin C is a water-soluble antioxidant vitamin that has been indicated to react with superoxide, hydroxyl radicals and singlet oxygen decreasing cell damage (Bowman, 2012). Moreover, Vitamin C simply passes through blood-brain barrier (Agus et al., 1997) and deactivates extracellular and intracellular free radicals, as it is a rich store of electrons which stick to free radicals to restrain their activity (Bendich, 1990).

Therefore, the aim of this study was to investigate the possible neurotoxic effects of prenatal and postnatal exposure to GA3 on the development of the cerebellum and the possible protective role of Vitamin C on these changes if present.

MATERIALS AND METHODS

Chemicals

Gibaifar (5 % Gibberellic acid, GA3) in powder form was purchased from Sigma Chemical Co. 4ml of GA3 (equivalent to 200 mg of GA3) were diluted with tap water until 1000 ml to obtain 200 ppm of GA3(Troudi et al., 2012).

Vitamin C (Ascorbic acid) in drop form was purchased from the pharmacy as a product of Unipharma (Chem. Co., Egypt). It was dissolved in normal saline solution (Dawson et al., 1999).

Experimental Animals and groups

The study was conducted at the Human Anatomy and Embryology Department, Faculty of Medicine, Suez Canal University.

A total of 25 male and 50 female healthy Sprague-Dawly rats, 10-12weeks of age, weighing 180-200 g, were used throughout the study. They were obtained from the Animal house of the Faculty of Veterinary Medicine, Suez Canal University. They were housed individually for a 2-week acclimatization period prior to the experiment. Rats were fed ad libitum by standard laboratory pellet and tap water. A 12-hr light, 12-hr dark cycle was maintained. Room temperature was at 23±2 ˚C with a relative humidity of 45-55%. All experimental procedures and ani-mal maintenance were conducted in accordance with the institutional standards of animal care and approved by the local ethics committee. After the 2 week-period of acclimatization, female rats during their pro-estrous phase of the estrous cycle were caged overnight with the males of the same stock (female: male=1:1). The vaginal smear was examined early in the next morning. Presence of spermatozoa in the smear was taken as day 'one' of pregnancy (GD1). Pregnant females were caged individually till term and were divided randomly into four equal groups:

Group I (Control group) were subdivided into: IA (negative control), where animals did not receive anything. IB (positive control), where animals received tap water via intra-gastric intubation from the 14th day of pregnancy until the day 14 after delivery as drinking water (Troudi et al., 2012).

Group II (GA3-treated group), where rats received a daily oral dose of 200 ppm of GA3 (equivalent to 55 mg/kg) via intra-gastric intubation from the 14th day of pregnancy until the day 14 after delivery. This used dose was chosen to provoke oxidative stress without lethal effects in suckling rats whose mothers were treated with GA3 (Troudi et al., 2012)

Group III (GA3 & Vitamin C-treated group), where rats received GA3 (equivalent to 55 mg/kg) simultaneously with 100 mg of Vitamin C/kg via intra-gastric intubation from the 14th day of pregnancy till day 14 after delivery (Troudi et al., 2012; Dawson et al., 1999).

Group IV (Vitamin C -treated group), where rats received a daily oral dose of 100 mg of Vitamin C / kg via intra-gastric intubation from the 14th day of pregnancy till day 14 after delivery (Dawson et al., 1999).

One month after delivery, pups from all groups were sacrificed and the cerebella were extracted. Half of the cerebella were processed for light microscopic examination while the other half was processed for transmission electron microscopic examination.

Light Microscopic Study

The collected cerebellar tissues were fixed in aqueous Bouin's fixative, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and then impregnated in paraffin wax. Sections of 5-7 µm thickness were taken and stained with Hematoxylin & Eosin (H&E), examined and photographed under light microscope (Bancroft and Gamble, 2008).

Transmission Electron Microscopic Study

Each cerebellum was cut into small pieces of about (1-3 mm^3) under a dissecting microscope and immediately fixed in 2.5% glutaraldehyde for 24-48 hr. These specimens were then washed in phosphate buffer (pH 7.2-7.4) 3-4 times for 20 min. every time and post-fixed in a buffered solution of 1% osmium tetraoxide for 2 hr., then washed in the same buffer 4 times for 20 min. each. Fixed speci-

mens were dehydrated in ascending grades of ethyl alcohol (30%, 50%, 70%, 90% and 100%), cleared in two changes of propylene oxide and embedded in Epoxy resin (Hayat, 2000). Semithin sections (1µm thick) were prepared and stained with toluidine blue, examined and photographed under the light microscope. Ultrathin sections (60–90 nm thick) were cut, mounted on copper grids and double-stained with uranyl acetate and lead citrate (Gupta 1983). These grids were examined and photographed using a transmission electron microscope (JEOL JEM-1010, Japan) operated at 60-70 kV, Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Morphometric study

The morphometric measures were performed by the touch count method using a computer assisted image analyzer (soft imaging system –An Olympus Company) at the Histology Department, Faculty of Medicine, Al-Azhar University. The measurements were performed using a ×40 objective lens in five non-overlapping fields in ten randomly chosen sections from six different animals for each group. The following parameters were studied:

Thickness of all layers of the cerebellar cortex (µm): molecular cell layer (MCL), Purkinje cell layer (PCL) and granular cell layer (GCL) (Razi et al., 2015).

Mean number and diameter (µm) of the Purkinje cells/ field (the number of Purkinje cells that have nuclei / 10000 µm^2 area of the Purkinje cell layer) (Razi et al., 2015).

Statistical analysis

The analysis was done with SPSS19.0. The morphometric data of each animal group were statistically analyzed and the ANOVA test was employed to compare the studied animal groups. Data were expressed as the mean (±) SD. Significance of the data was determined by P values where a P< 0.05 was considered significant.

RESULTS

Light Microscopic results

Light microscopic examination of the H&E stained sections of the cerebellar cortex of the control and Vitamin C groups revealed normal architecture. The grey matter of the cerebellar cortex was formed of three layers; outer MCL, middle PCL and inner GCL.

The outer MCL has sparse population of neurons. The PCL shows large flask-shaped Purkinje cells arranged in a single row at the junction of the MCL with the GCL. These cells displayed a characteristic centrally located rounded open-face nucleus, with prominent nucleolus and surrounded by Bergmann astrocytes scattered in the superficial part of the granular cell layer and in-between the Purkinje cells with multiple shapes having pale nuclei and pale cytoplasm. The GCL contained numerous compactly arranged granular nerve cells with darkly stained nuclei surrounded by very little cytoplasm (Fig. 1).

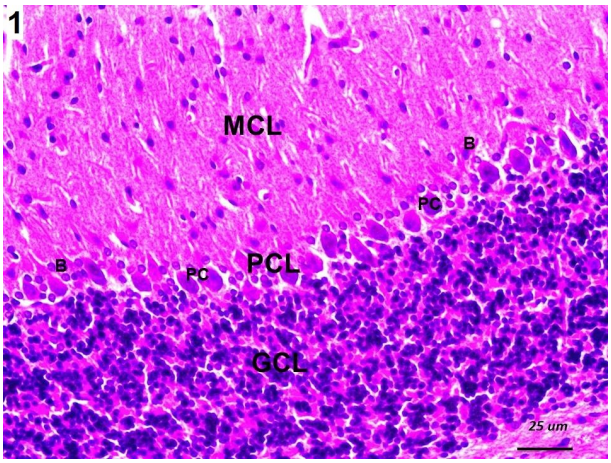

Fig 1. A Photomicrograph of a section of the cerebellar cortex of the control group showing the outer molecular layer (MCL), the middle Purkinje layer (PCL) having large flask-shaped Purkinje cells (PC) with centrally located large vesicular nuclei and surrounded by Bergmann astrocytes (B). The inner granular layer (GCL) composed of closely packed deeply stained numerous cells (H&E, x400).

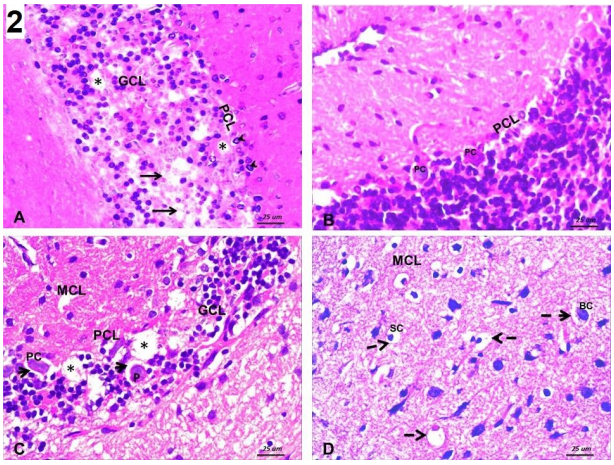

Fig 2. Sections of the cerebellar cortex of GA3-treated group. A. marked distorsion of Purkinje cells (PCL) with few astrocyes (head arrows). Granular cell layer (GCL) showing many vacuoles (asterisks) with areas of patchy cell loss (black arrows). B. shrunken Purkinje cells (PC) with an eosinophilic cytoplasm and fragmented nuclei. C. disturbed shrunken Purkinje cells (PC) being flat with dark cytoplasm and surrounded by halos of empty space (arrow head), some appeared displaced downward (P) in granular cell layer (GCL). Many vacuoles appear (asterisk). D. the molecular layer (MCL) with shrunken basket cells (BC) and stellate cells (SC) being surrounded by prominent perineural spaces (detached arrows). (H&E, x400).

The cerebellar cortex of GA3-treated rats (Group II) revealed marked histological alterations markedly on the PCL. The Purkinje cells appeared distorted, disarranged with irregular size and shape. Few astrocyes were noticed. The GCL showed many vacuoles with areas of patchy cell loss (Fig. 2A). Few shrunken Purkinje cells with an eosinophilic cytoplasm and fragmented nuclei were seen (Fig. 2B). Disturbed shrunken displaced Purkinje

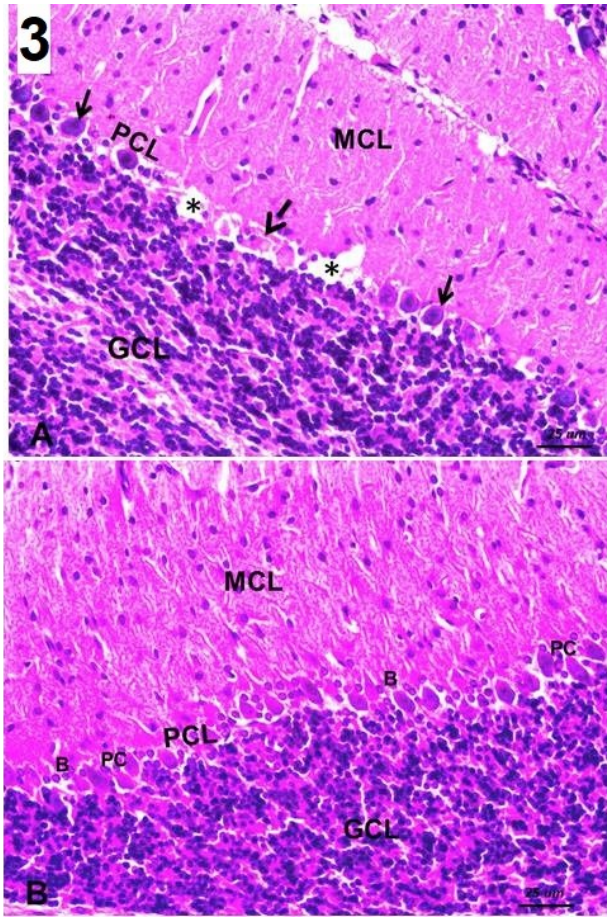

Fig 3. Sections of the cerebellar cortex of GA3 & Vitamin C-treated group. A. Relatively normal linear appearance of Purkinje cells layer (PCL). Purkinje cells regain their normal flask shape (arrows) with darkly stained nuclei and cytoplasm. Few vacuoles (asterisks) and degenerated Purkinje cells (dashed arrow) are still seen cells. Both molecular (MCL) and granular cell layers (GCL) appeared normal. B. Normal linear appearance of Purkinje cells layer (PCL) with greater numbers of normal flask shaped Purkinje cells (PC) with aggregations of excess astrocyes around them (B) are noticed. Molecular (MCL) and granular cell (GCL) layers appeared as control group (H&E, x400).

cells in the granular layer with dark cytoplasm and surrounded by halos of empty space were also noticed (Fig. 2C). The MCL appeared with shrunken basket and stellate cells being surrounded by prominent perineural spaces (Fig. 2D).

The cerebellar cortex of the rats that received GA3 simultaneously with Vitamin C (Group III) revealed restoration of the normal appearance of all layers of cerebellar cortex to the control group. Relatively normal linear appearance of the PCL was seen and most of the Purkinje cells regained their flask shape with darkly stained nuclei and cytoplasm. However, a small number of cells appeared degenerated and others disappeared leaving empty spaces (vacuoles) (Fig. 3A). Some astrocyes were aggregated around normal Purkinje cells (Fig. 3B). Both MCL and GCL appeared as control group (Fig. 3 A,B).

In semithin sections stained with toluidine blue the control group revealed that the Purkinje cells

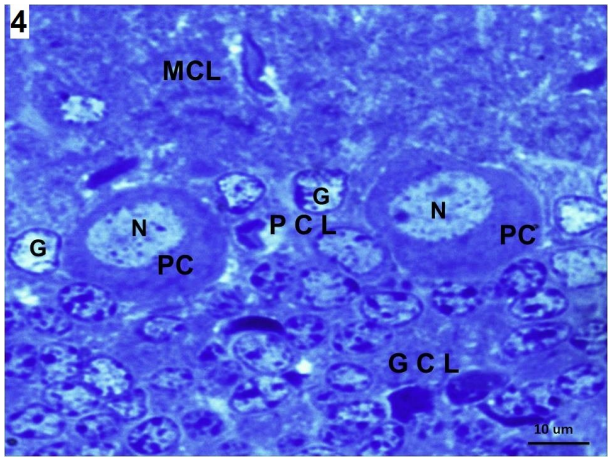

Fig 4. Semithin section of the cerebellar cortex of the control group showing normal molecular layer (MCL), normal Purkinje cell layer (PCL), and normal granular layer (GCL). Purkinje cell (PC) is seen arranged in one row along the outer margin of the GCL. These neurons have pale nuclei (N) and surrounded by glial cells (G) (Toluidine blue, x1000).

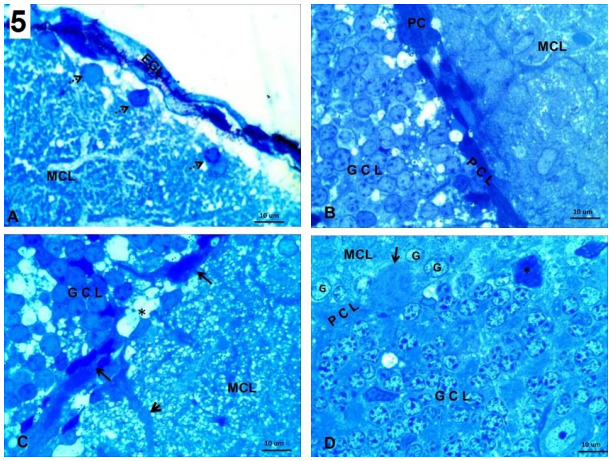

Fig 5. Semithin sections of the cerebellar cortex of GA3-treated group. A. presence of external granular layer (EGL) with detached granule cells (detached arrows) external to the molecular cell layer (MCL). B. few abnormal flat Purkinje cells (PC) with darkly stained nuclei and cytoplasm in the Purkinje cells layer (PC) in-between the granular and molecular cell layers (GCL) and (MCL). C. distorted irregular Purkinje cells (arrows) with dark stained cytoplasm and non-defined nuclei. Vacuoles are seen (*). The molecular cell layer (MCL) showed a dendrite (arrowhead). D. distorted Purkinje cell layer (PCL) with loss of linear appearance. Purkinje cell appeared either large, irregular with homogenous cytoplasm (arrow) and surrounded by swollen glial cells (G) or rounded with dark stained cytoplasm (asterisk). (Toluidine blue, x1000).

appeared in linear position between the GCL and MCL with rounded shape, large pale nucleus, prominent deeply stained nucleolus and basophilic cytoplasm. Both the GCL and MCL appeared normal, their nuclei contained dense clumps of chromatin at the nuclear membrane (Fig. 4).

The cerebellar tissues of GA3-treated group revealed the presence of the external granular layer (EGL) as a thin layer external to the MCL with detached granular cells (Fig. 5A). Few abnormal flat Purkinje cells with darkly stained nuclei and cyto-

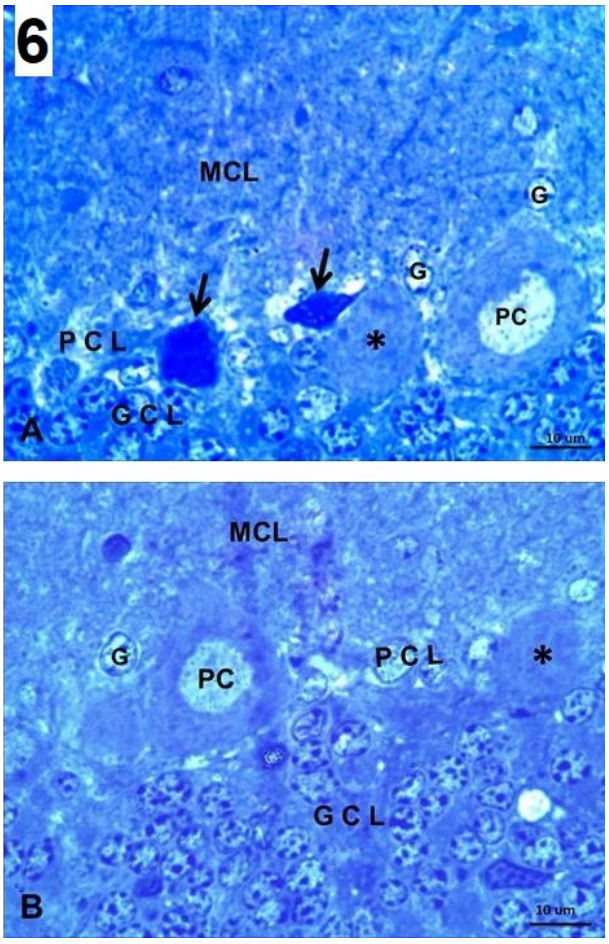

Fig 6. Semithin sections of the cerebellar cortex of Group III (GA3 & Vitamin C). A. relative restoration of the normal linear position of Purkinje cells layer (PCL). Purkinje cells regained its normal shape (PC) and surrounded by glial cells (G). Purkinje cell with homogenous cytoplasm and ill-identified nuclei (asterisk) and shrunken Purkinje cell with deeply stained cytoplasm are still seen (arrows). Molecular cells layer (MCL) and granular cells layers (GCL) are as control. B. Normal linear position of Purkinje cells layer (PCL) with normal Purkinje cell (PC) surrounded by glial cells (G). Notice, one Purkinje cell with homogenous cytoplasm and ill-identified nuclei (asterisk) is still present. Both molecular cells layer (MCL) and granular cells layer (GCL) are normal. (Toluidine blue, x1000).

plasm in-between the granular and molecular cell layers (Fig. 5B) were noticed in addition to distorted irregular Purkinje cells with dark stained cytoplasm and non-defined nuclei accompanied by vacuolations. The molecular cell layer showed a dendrite (Fig. 5C)**.** Distorted Purkinje cell layer with loss of linear appearance and the Purkinje cell appeared either large, irregular with homogenous cytoplasm and surrounded by swollen glial cells, or rounded with dark stained cytoplasm (Fig. 5D).

The cerebellar cortex of GA3- & Vitamin C-treated group showed improvement and restoration of the normal structure of the cortical layers of the cerebellum. PCL was seen as normal in linear position between GCL and MCL. Most of the Purkinje cells regained the normal appearance and greatly resembled the shape of the control

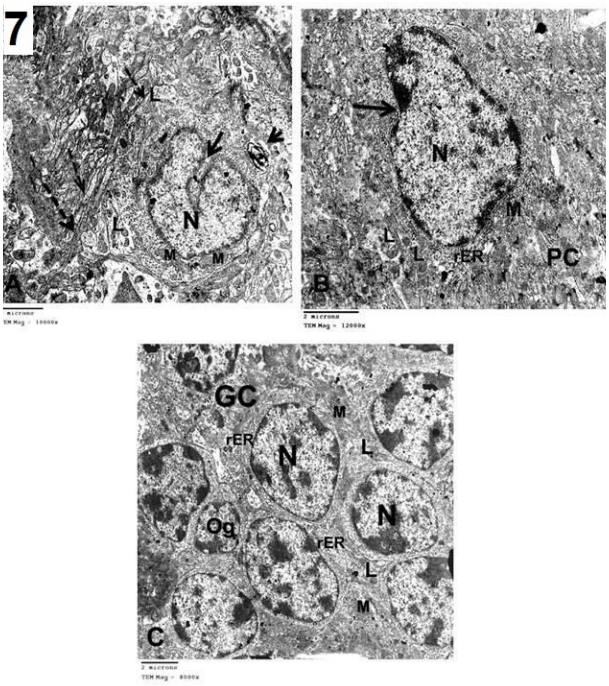

Fig 7. Electron micrographs of cerebellar cortex of the control group. A. the molecular layer containing Basket cell with euchromatic nucleus (N) and invagination of its nuclear envelope (arrow), the cytoplasm contains mitochondria (M), lysosomes (L). There are many dendrites (dashed arrows) in the surrounding neuropil and synapses (arrowhead). (x 10,000). B. the Purkinje cell (PC) with euchromatic nucleus (N) and invaginated nuclear envelope (arrow). Its cytoplasm contains rough endoplasmic nucleus (rER), lysosome (L) and numerous mitochondria (M) (x 12,000). C. the granular cells (GC) crowded in groups with central large oval nuclei (N), peripheral clumped chromatin and surrounded by thin rim of cytoplasm. The cytoplasm contains mitochondria (M), rough endoplasmic reticulum (rER) and lysosomes (L). Oligodendrocytes (Og) are present between the granular cells (x 8000).

ones and were seen surrounded by glial cells. However, few cells appeared either having homogenous cytoplasm with ill-identified nuclei or shrunken with deeply stained nuclei and cytoplasm. Both GCL and MCL appeared as the control group. The external GCL was absent (Fig. 6 A,B).

Electron Microscopic results

Ultrathin sections of the cerebellar cortex of the control and Vitamin C treated group revealed that the MCL is formed of an extension of axons and dendrites of cells of the same molecular layer and dendrites of the Purkinje cells. The basket cells were widely distant from each other and contained a small amount of cytoplasm surrounding the pale euchromatic nuclei with nuclear envelop invagination. The cytoplasm of these cells contained few dense small-sized mitochondria and short cisternae of rough endoplasmic reticulum (Fig. 7A). The Purkinje cells were irregular in shape and contained large eccentric euchromatic nuclei and nucleoli bounded by cytoplasm containing rough endoplasmic reticulum, well-defined mitochondria and lysosomes. The Purkinje cells and their nuclei

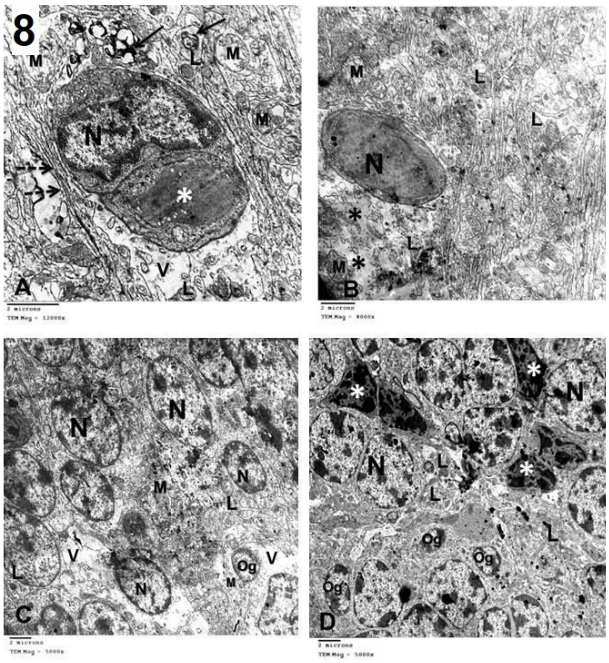

Fig 8. Electron micrographs of cerebellar cortex of GA3 treated rats. A. the molecular layer containing abnormal basket cell with irregular nucleus and deformed chromatin (N), accompanied with degenerated cytoplasm (white strike). The basket cell was surrounded by vacuolation (V), enlarged active lysosomes (L) and swollen mitochondria (M). Abnormal synapses (arrows) and thin dendrites (detached arrows) were also seen. (x 12,000). B. Purkinje cell with abnormally degenerated nucleus surrounded by degenerated cytoplasm and area of oedema (asterisk). There are swollen mitochondria (M) and hyperactive lysosomes (L) (x 8000). C&D. the granular cells with abnormal variable sized nuclei with coarse chromatin (N), some surrounded with vacuolated cytoplasm (V), degenerated mitochondria (M) and swollen lysosomes (L). Condensed and degenerated oligodendrocytes (Og), either with electron dense clumped chromatin or pyknotic nuclei with apoptotic changes (white asterisk) are seen. (x 5000).

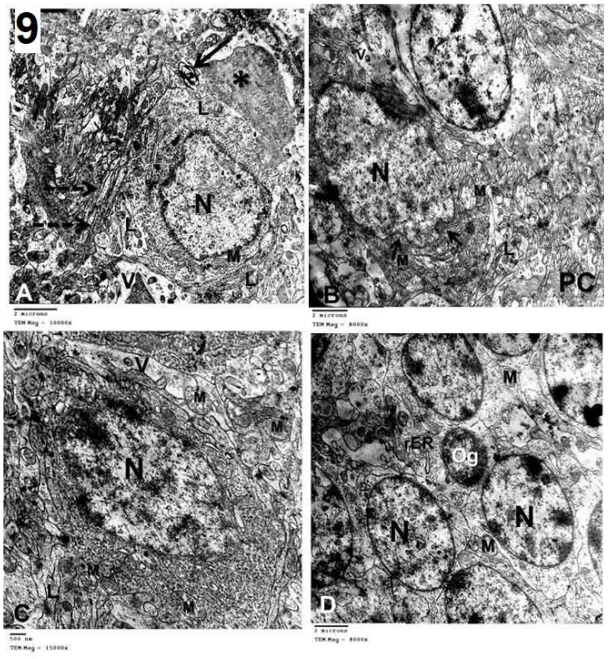

Fig 9. Electron micrographs of cerebellar cortex of rats treated GA3 and Vitamin C. A. the molecular layer; the basket cells appear normal with slightly rounded nuclei (N) and condensed chromatin. It is surrounded by normal cytoplasm containing slightly swollen mitochondria (M) and lysosomes (L). Fewer synapses are present (arrow) and little dendrites (detached arrows). Area of degeneration (black strike) and vacuolation are present in surrounding neutrophil (V) (x 10,000). B. Purkinje cells (PC) appearing normal with irregular nuclei (N), condensed clumped nuclear chromatin and invaginated envelope (arrows). Their cytoplasm shows active mitochondria (M) and excess lysosomes (L). Some vacuolation (V) are still present in the surrounding neuropil (x 8000). C. Purkinje cell appears with active slightly constricted nuclei with condensed cytoplasm containing swollen mitochondria (M), lysosomes (L) and surrounded by small area of vacuolation (V) (x 5000). D. shows the granular cells, which appear similar to those of the control group with rounded nuclei (N), active cytoplasm with excess mitochondria (M), rough endoplasmic reticulum (rER) and oligodendrocytes (Og) in between (x 8000).

contained large blocks of condensed chromatin distributed on the inner side of the nuclear envelope, which showed slight invagination (Fig. 7B). The GCL contained many dark small granular cells, having slightly dark oval nuclei with characteristic condensed chromatin surrounded by very little cytoplasm containing rough endoplasmic reticulum, mitochondria and lysosomes. Oligodendrocytes were scattered between the granular cells (Fig. 7C).

The cerebellar cortex of the rats of the GA3-treated group revealed observable alterations. Most cells of the MCL were degenerated with patchy loss. The dendrites of their cells and those of Purkinje cells were degenerated. Degenerated basket cells with shrunken nuclei and absence of nucleoli were seen. Their cytoplasm showed vacuolations accompanied with degenerated mitochondria, enlarged lysosomes, vacuolated neutrophil with loss of matrix and few synapses (Fig. 8A). Most of the Purkinje cells appeared deformed and shrunken. Some nuclei of the Purkinje cells were pyknotic, accompanied with vacuolated cytoplasm and disrupted abnormal swollen or degenerated mitochondria (Fig. 8B). In the GCL, the granular cells appeared with variable sizes: some cells appeared with condensed nuclear chromatin with irregular nuclear envelopes, while others showed pyknotic nuclei. Their cytoplasm had disrupted swollen mitochondria. The oligodendrocytes of the granular layer showed condensation, degeneration, either with electron dense clumped chromatin or pyknotic nuclei with apoptotic changes (Fig. 8 C,D).

Concerning the group that received GA3 simultaneously with Vitamin C (Group III), the cerebellar cortex showed a histological picture near to the normal one. The nuclei of the basket cells of the MCL were found to be rounded with condensed chromatin. However, few swollen mitochondria and lysosomes were still seen. Synapses were retained (Fig. 9A). Most of the Purkinje nerve cells appeared normal and their nuclei contained blocks

Table 1. Mean ± SD of the morphometric parameters among the different groups

Morphometric parameter	Group I - Control group	Group II - GA3 treated group	Group III - GA3 & Vitamin C group	Group IV - Vitamin C treated group
Thickness of MCL(µm)	24.6 ± 4.6	16.8 ± 3.7*[a]	21.4 ± 6.2*[b]	22.6 ± 3.2*[b]
Thickness of PCL(µm)	5.3 ± 1.3	1.5 ± 0.7*[a]	2.7 ± 0.7*[a,b]	6.1 ± 1.7*[b]
Thickness of GCL(µm)	84.7 ± 12.3	54.6 ± 13.2*[a]	63.7 ± 16.2*[a,b]	87.3 ± 11.8*[b]
Number of PC (PC number /10000 µm² area of PCL)	12.3 ± 2.8	4.3 ± 0.8*[a]	8.6 ± 1.6*[a,b]	11.5 ± 1.8*[b]
Diameter of PC (µm)	8.6 ± 1.7	3.2 ± 0.7*[a]	6.3 ± 0.9*[a,b]	9.2 ± 2.1*[b]

*P≤0.05
a compared to the control group.
b compared to the GA3 treated group.

of condensed chromatin distributed on the inner side of the nuclear envelope with slight invagination. Its cytoplasm showed activated lysosomes and mitochondria. Vacuolation was still seen surrounding neutrophils <(Fig. 9 B,C). The granular cells were almost similar to those of the control group. However, very few cells still showed active cytoplasm with excess mitochondria and active rough endoplasmic reticulum (Fig. 9D).

Morphometric results

A statistically significant decrease in the thicknesses of the MCL, PCL and GCL in addition to the number and diameter of the Purkinje cells was evident in the GA3-treated rats (Group II) when compared with the control one. Administration of Vitamin C resulted in a significant increase in the thicknesses of these layers when compared to GA3-treated group. However, a statistically significant decrease was still found when compared with the control group (Table 1).

DISCUSSION

From the present findings, pre- and post- natal maternal treatments with GA3 led to the development of morphological alterations in the cerebellar cortex of their pups. Most of these alterations were noticed in the Purkinje cell and granular cell layers. These current results are in accordance with those reported by (Neil and Reece, 2002; Schwechheimer and Willige, 2009; Allam et al., 2011; Troudi et al., 2012; Abou-zeid and Abd-Ellah, 2015).

Many theories have been developed to explain the mechanism of action of GA3. According to Furukawa et al. (2004) and Yilmaz and Celik (2009), the central nervous system is more susceptible to oxidative damage of PGRs due to its high oxygen consumption rate and relatively low levels of defense mechanisms against toxicity. Samson and Nelson (2000) mentioned that the central nervous system has high levels of polyunsaturated fatty acids, found to facilitate oxidative damage. Moreover, the antioxidant effect induced by GA3 may have resulted mainly from depletion of the antioxidant enzymes system (Kroemer, 2003), which led to the impairment of homeostasis and reducing cellular defense system against toxicity originated by the liberation of active oxygen forms (Oruc and Uner, 2002). Therefore, the prominent alterations observed in the cerebellar cortex of offspring of GA3-treated group could be caused by oxidative stress, which acts as a contributor to the initiation or progression of cellular damage by inducing the reactive oxygen species (ROS) that oxidize vital cellular components such as lipids, proteins and DNA, producing potentially harmful effects.

In parallel, the current observed changes in the Purkinje cells might be also due to the elaboration of free radicals of GA3 that led to reduced Purkinje cell proliferation, differentiation, and maturation with increase in Purkinje cells' death. Sobaniec-Lotowska (2001) suggested that the marked changes in the mitochondria of Purkinje cells could be interpreted as a disorder of the intercellular biochemical events, including inhibition of oxidative phosphorylation, due to direct toxic effect of the drug or its metabolites. Moreover, Olivi et al. (1993) reported that dying neurons can undergo condensation and dissolution of chromatin. Hence, the observed vacuolated cytoplasm, degenerated mitochondria, vesiculated rough endoplasmic reticulum, vacuolated neutrophil, loss of matrix and few synapses might be due to the increased amount of ROS product, which attack membranes and enhance their permeability leading to vacuolization, which is the primary response to cell injury (Jacquard, 1968).

It has been previously explained that the observed condensed nuclear chromatin, irregular and indented nuclear envelopes, and pyknotic nuclei in the GCL accompanied with dilated cisternae of rough endoplasmic reticulum and disrupted, very swollen mitochondria occur in early stages of apoptosis and may precede and/or accompany nuclear changes (Yu et al., 2008). Additionally, the condensed and degenerated oligodendrocytes with apoptotic morphology may be caused by GA3 free radicals that may block neuronal activity, causing the neurons to receive internal signals to commit suicide (apoptosis) (Lin and Beal, 2006). Another possible mechanism is that oxidative stress may lead to membrane degradation, cellular dysfunction, and finally, apoptosis (Catalá, 2007).

Therefore, the observed changes in the current study might reflect the association between GA3 and oxidative stress. The antioxidant defense system maintains a relatively low rate of the reactive

and harmful OH (Regoli and Principato, 1995). The free radical oxygen has a potential role in neural cell damage. Exogenous antioxidant such as Vitamin C can be effective on neuronal cell protection due to the effect of ROS on neuronal cell damages and fast consumption of endogenous scavenging antioxidants (Sánchez-Moreno et al., 2003; Varshosaz et al., 2014).

In the current study, administration of Vitamin C to GA3-treated mothers resulted in improvement of the histological cerebellar alterations seen with GA3-treated group. However, slight alterations were still seen in the PCL. This denotes that Vitamin C neuro-protective sensitivity of the Purkinje cell is less than other cerebellar cells due to the effect of ROS, especially in PCL as excess glial cells which are particularly sensitive to free radicals. This is in accordance with other studies that revealed that repeated administration of ascorbic acid significantly attenuated ischemic damage induced by circulatory disturbances in the brain cortex in rats with experimental cerebral ischemia (Shokouhi et al., 2004). This is also in match with Farombi and Onyema (2006), who mentioned that dietary antioxidants such as vitamins C and E and quercetin had protective potential against oxidative stress induced by food supplements as monosodium glutamate (MSG), and also by Pavlovic et al. (2007), who found that treatment with ascorbic acid may prevent the MSG-induced cytotoxicity in rat thymocytes by up-regulating Bcl-2 protein expression.

These results are also in accordance with Afifi and Embaby (2016), who reported a protective role of ascorbic acid on cadmium-induced cerebral neurotoxicity in rats, and with Varshosaz et al. (2014), who also confirmed the protective role of ascorbic acid on ischemic cerebellar damage in rats.

It has been suggested that Vitamin C exerts a neuro-protective action through scavenging the oxygen-free radicals (Peng et al., 2005). Moreover, it has been added that through decreasing lipid peroxidation and increasing catalase activities, Vitamin C exerts neuro-protection (Jetti et al., 2014). Additionally, ascorbic acid showed antiapoptotic effect through decreasing Bax protein, enhancing Bcl-2 protein (Han et al., 2007), and exerted neuroprotective action through decreasing lipid peroxidation and increasing catalase activities (Han et al., 2007; Santos et al., 2008).

Furthermore, the antioxidant mechanism of Vitamin C has been mentioned to be based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen (Sisein, 2014; Lee et al., 2004). Scavenging aqueous radicals and regeneration of tocopherol from the tocopheroxyl radical species are also well-known antioxidant mechanisms of Vitamin C (Sisein, 2014; Lee et al., 2004).

Additionally, it has been mentioned that the neuro-protective effect of Vitamin C may be due to its ability to cross the blood brain barrier (Peng et al., 2005). Moreover, Vitamin C contributed to the synthesis of the amino acids; carnitine and catecholamines regulating the nervous system functions (Grosicki, 2004).

Conclusion - The results of the current study showed that maternal exposure of rats to GA3 during late pregnancy and lactating period provoked degenerative changes in the cerebellar cortex of rats' offspring, as evidenced by multiple histological alterations, especially in Purkinje cell and granular cell layers. With Vitamin C administration, these degenerative changes were minimized.

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