



Experimental Study

Histological Study on the Protective Role of Ascorbic Acid on Cadmium Induced Cerebral Cortical Neurotoxicity in Adult Male Albino Rats

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ABSTRACT

Cadmium (Cd) toxicity represents a worldwide problem in environmental contamination and a common cause of occupational and non-occupational neurological diseases. So, this study aimed to evaluate the histological changes induced by Cd on the cerebral cortex of adult rats and evaluating the possible ameliorating role of ascorbic acid (AA). Twenty adult male rats were divided into; control group, AA group (each rat was received a daily oral dose of 200 mg AA/kg body weight (b.w) and Cd group (each rat was received 5 mg Cd/kg b.w orally) and protective group (each rat was given AA concomitantly with Cd at the same dose, route and period of administration of the previous groups. After two months the cerebral cortexes were processed for histological examination. The cerebral cortex of Cd treated animals exhibited severe degenerative changes especially in pyramidal and granule cells. Structural changes in these cells were in the form of dilated rER and Golgi complex, swollen mitochondria and marginated nuclear chromatin. Myelinated nerve fibers displayed myelination disruption and irregular neurofilaments. The neuropil appeared vacuolated with accumulation of neuroglial cells. On the other hand, these changes were ameliorated in rats which received AA concomitantly with Cd. So, it could be concluded that AA can ameliorate the histological changes induced by Cd and this direct the attention to the antioxidants as protective measures for the neurotoxicity.

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1. Introduction

Cadmium (Cd) is a naturally occurring heavy metal posing severe risks to human health. It is known to be one of the most toxic environmental and industrial pollutants that cause water, air and food pollution [1]. Human exposure to Cd occurs chiefly through inhalation or ingestion. Ten to fifty percent of inhaled cadmium dust and about five to ten

percent of ingested Cd is absorbed depending on its particle size [2].

Cd is unique among other metals because of its toxicity at a very low dosage, long biologic half life and its low rate of excretion from the body. Commercially, it is used in television screens, lasers, batteries, paint pigments, cosmetics, galvanizing steel, copper alloys, rubber and plastics stabilizers, fungicides and in many other products [3].

Food, including vegetables, is also an important source of entry of Cd and populations such as subsistence farmers that consume locally grown products are at particular risk [4]. Cd has been found also in beverages, fish, meat, milk, eggs, cereals and also present in cigarettes. Therefore,

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major sources of Cd intake in human beings are food or smoking [5].

People who live near hazardous waste sites or factories that release Cd into the air have been shown to suffer from impaired health, such as damaged lungs, bone fracture, reproductive failure and possibly infertility [6]. In addition, psychological disorder, damage of central nervous system and DNA or cancer development can also occur [7].

Cd exerts its toxic effects via oxidative damage to cellular organelles by inducing the generation of reactive oxygen species (ROS) which react with cellular biomolecules leading to lipid peroxidation, membrane protein damage, altered anti-oxidant system, altered gene expression and apoptosis [8].

As oxidative stress is one of the important mechanisms of cadmium-induced damages, it can be expected that the administration of some antioxidants should be an important therapeutic approach [9].

Ascorbic acid [AA] is one of the water-soluble antioxidants that present in citrus fruits, vegetables and strawberries [10]. It has a protective role against Cd-induced histological changes in liver, kidney, lungs and testis, as well as the cytotoxicity in bone marrow in rats. It also showed neuro-protection against ischemia, sciatic nerve injury in rats [11] and ethanol induced toxicity [12]. It also attenuated the lead induced apoptosis in hippocampus [13].

As Cd pollution is generally wide spread, this work aimed to study histologically and morphologically the possible protective role of AA against the Cd induced changes in the cerebral cortex of adult male albino rats using light and transmission electron microscopes.

2. Materials and Methods

The study was conducted at the Animal House of Kasr El Aini, Faculty of Medicine, Cairo University, according to the guidelines for the care and use of laboratory animals.

A total of twenty male albino rats 5–6 months old, weighing 200–220 g constituted the animal model in this study. Each group was housed in a separate cage (Suzhou Suhang Technology Equipment Co., Ltd.) in a constant temperature (22–24 °C) and light-controlled room on an alternating 12:12 h light-dark cycle and had free access to food and water. Rats were fed a standard commercial pellet diet and were kept for one week before beginning the experiment for acclimatization.

2.1. (A) Experimental Design

The animals were randomly divided into four groups (five rats each): group I: served as control and was given only distilled water. Group II (ascorbic acid group): each animal was received a daily oral dose of 200 mg ascorbic acid/kg b.w (Sigma Chemical Co.: St. Louis, MO, USA) dissolved in distilled water according to [14]. Group III (Cadmium received group): each rat was received 5 mg Cd/kg b.w (Merck, Darmstadt, Germany) orally and daily dissolved in water according to [3]. Group IV (protective group): each rat was given AA at the same dose, period and route of administration of the group II concomitantly

with Cd at the same dose and route of group III. The oral route administration was chosen as it is the main mode of exposure to Cd in humans and animals [15].

After two months, the rats were anaesthetized with 4% halothane and perfused with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde solution [16]. After perfusion, cerebral hemispheres from each animal were dissected out, coronal section was done and specimens were taken from the frontal lobe and processed for light and electron microscopic study.

2.2. (B) Light Microscopy

The specimens were fixed in 10% neutral- buffered formalin, processed for preparation of paraffin section. 5 µm thicknesses sections were cut with a microtome (Leica RM 2025, Germany) mounted on glass slides and stained with hematoxylin and eosin (H&E) for general examination [17].

2.3. (C) Electron microscopy

Very small pieces were processed and embedded in Epon. Semithin sections were cut, stained with toulidine blue and examined by light microscope to choose the selected areas. Ultrathin sections were cut with Leica ultracut (Leica Ltd, Glienicke, Berlin, Germany) using diamond knife on copper grids and stained with uranyl acetate followed by lead citrate [18]. Finally, each grid was examined and photographed using JEOL-JEM-100 SX electron microscope, Japan at 80- kilo vol (Jeol Ltd, Tokyo, Japan) at EM unit in Tanta University.

2.4. (D) Morphometric Statistical Study

Leica Qwin 500 LTD (Cambridge, UK) computer assisted image analysis was performed in Histology Department, Faculty of Medicine, Cairo University. The percent of vacuolated cells with dark nuclei were estimated in ten randomly chosen high power fields (HPFs) using interactive measurements menu. The mean values were obtained and compared between the different groups. Data were analyzed using SPSS by Student's t-test. The results were considered non-significant when $p > 0.05$ and highly significant when $p < 0.001$.

3. Results:

3.1. Light microscopic results

Microscopic examination of H&E stained sections of the cerebral cortex from frontal area of group I (control) and group II (ascorbic acid group) were similar and showed the well known normal structure of the cerebral cortex. They revealed six layers from outside inwards. The common cells inside these layers are the neurons especially pyramidal and granule cells in addition to neuroglial cells. The pink stained background; the neuropil, was a mat of neuronal and glial cell processes (Fig. 1).

Examination of H&E stained sections obtained from cadmium received group (group III) revealed severe

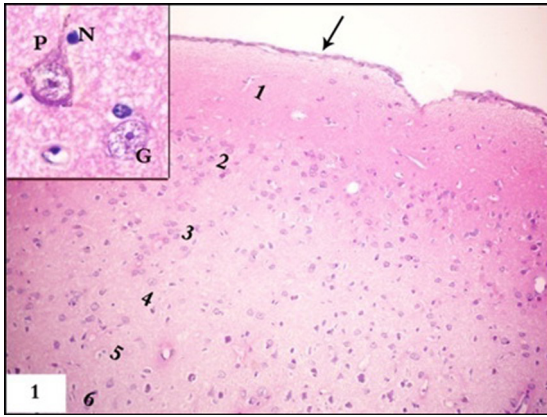


Fig. 1. A photomicrograph of a section in the cerebral cortex of control adult rat showing the general histological structure of the cerebral cortex. Notice, pia matter (arrow), molecular layer (1), outer granular layer (2), outer pyramidal layer (3), inner granular layer (4), inner pyramidal layer (5) and the multiform layer (6) (H&E 100X). Inset showing pyramidal cell (P) and granule cell (G) with pale open face nucleus and neuroglia (N) with dense nuclei (H&E, 1000 X).

multifocal histological changes in all layers of the cortex as compared to the control group. Many vacuoles of variable sizes either single or multiple appeared between and inside most of cells in all layers (Fig. 2 a). Some areas became more

cellular especially in outer granular and outer pyramidal layer (Fig. 2 b) while others were less crowded with cells (Fig. 2 c). Most of nerve cells were shrunken with loss of their processes and had pericellular halos. The pyramidal cells were more affected; they lost their processes and had deeply stained nuclei and became irregular in shape. The neuropil among the nerve cells and neuroglia showed vacuolation (Fig. 2 c). Regarding the inner pyramidal layer, the pyramidal cells were more or less still pyramidal in shape, but they had deeply stained nuclei. Most of granule cells were affected in this layer and became faintly stained, ill-defined boundaries with loss of their nucleoli. Both cells had pericellular halos and showed vacuolated background (Fig. 2 d).

Examination of specimens obtained from animals treated with cadmium concomitantly with ascorbic acid for two months showed improvement in nerve cells in many areas (Fig. 3 a). Most of pyramidal and granule cells were more or less as that of control group. Hardly ever granule cells appeared as cell ghosts that lost their nuclei and were faintly stained. Some neuroglial cells were nearly as in the control animals, while few cells were more darkly stained and the neuropil was still vacuolated (Figs. 3 b, 3 c). Occasional pyramidal cells were still affected in between the normal granule cells. They were shrunken, had darkly stained nuclei and pericellular halos and the neuropil was still vacuolated (Fig. 3 d).

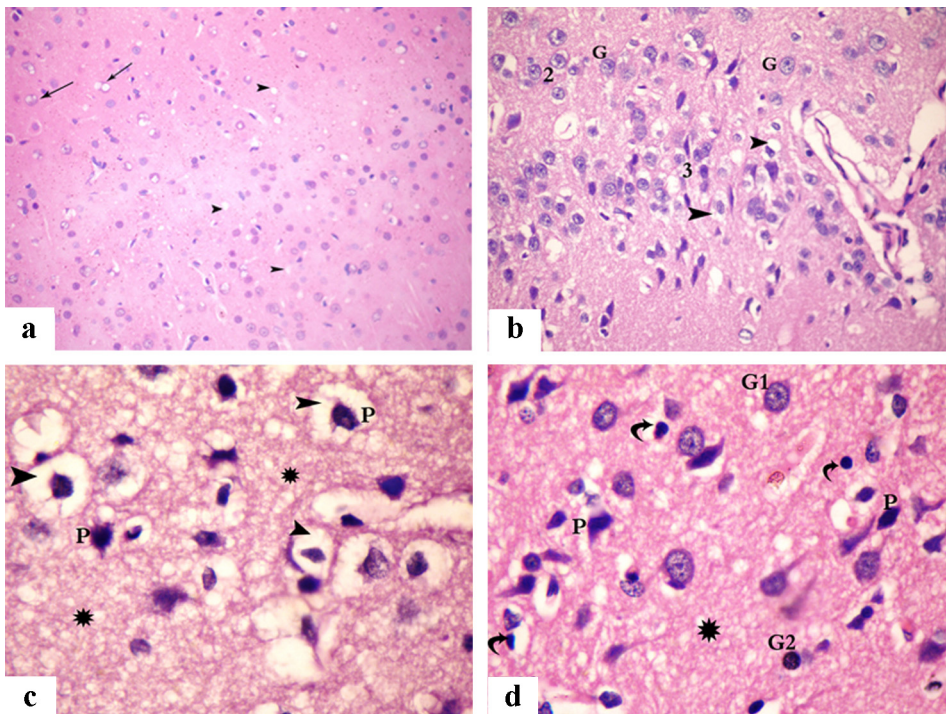


Fig. 2. Photomicrographs of a section in the cerebral cortex of a cadmium treated rat; (2a) showing intracellular (arrow) and extracellular vacuoles (arrow head) (H. &E. 400X). (2b): showing the outer granular (2) and outer pyramidal layers (3) crowded and become more cellular. Most of the granule cells are more or less normal while the pyramidal cells are irregular in shape and surrounded by pericellular halos (arrowhead). (H.&E. 400X). (2c): showing the outer pyramidal layer. Most of pyramidal cells are irregular in shape and have darkly stained nuclei (P). They have pericellular halos (arrow head) and the surrounding neuropil is also vacuolated (*). (H. &E. 1000X). (2d): showing large irregular pyramidal cells (P), deeply stained neuroglial cells (curved arrow) and vacuolated neuropil (*). Notice some granule cells with rounded nuclei and prominent nucleoli (G1), and few of them are shrunken, deeply stained (G2). (H. &E. 1000X).

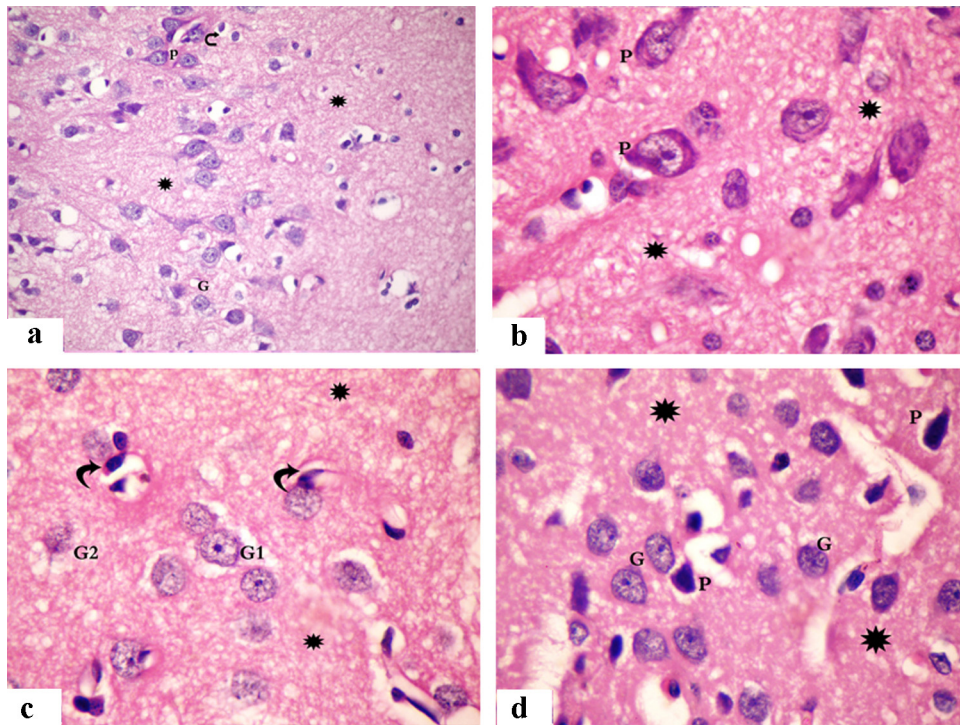


Fig. 3. Photomicrographs of a section in the cerebral cortex of a cadmium and ascorbic acid treated rat; (3a): showing more or less normal pyramidal cells (P) and granule cells (G). They have open face nuclei and basophilic cytoplasm. Notice apparently normal neuroglial cells (curved arrow) and vacuolated neuropil (*). (H. &E. 400X). (3b): showing nearly normal pyramidal cells with open face nuclei and basophilic Nissl granules (P). Notice the vacuolated surrounding neuropil (*). (H. &E. 1000X). (3c): showing granule cells with open face nuclei and prominent nucleoli (G1), while others appears as ghosts of nerve cells that lost their nucleoli and are faintly stained (G2). The neuroglial cells are darkly stained (curved arrow) and the surrounding neuropil is vacuolated (*). (H. &E. 1000X). (3d): showing normal granule cells (G) and darkly stained pyramidal cells (P). The surrounding neuropil is still vacuolated (*). (H. &E. 1000X).

3.2. E/M results

By transmission electron microscope, the cerebral cortex did not demonstrate substantial differences among rats in control and ascorbic acid-treated groups and it was similar to the well-known normal ultrastructural picture. The nerve cells had a large rounded euchromatic nuclei and were rich in rough endoplasmic reticulum (rER), polyribosomes, mitochondria and multiple perinuclear Golgi complexes (Fig. 4 a). Their axoplasm contained mitochondria as well as regularly arranged microtubules and neurofilaments (Fig. 4 b). The surrounding neuropil showed nerve fibers, synapses, neuroglial cells and blood capillaries. The nerve fibers either unmyelinated or myelinated were multiple and the myelinated fibers had regular compact myelin sheath (Fig. 4 c). The neuroglia had dense cytoplasm and irregular nuclei and their processes enveloped the blood capillaries with a continuous endothelial cell layer (Figs. 4 c, 4 d).

The ultrathin sections of nerve cells of Cd treated group showed dilatation of rER and perinuclear space, and swollen mitochondria with destroyed cristae. The nuclei were irregular in outline but exhibited euchromatin with scattered areas of dense chromatin (Figs. 5 a, 5 b). Most of nerve cells had small nuclei with very dense chromatin and vacuolated mitochondria (Fig. 5 c). The neuropil showed many affected nerve fibers either myelinated or

not. The myelinated fibers revealed areas of degenerative changes in the form of disruption, splitting, and loss of the lamellar compact structure of myelin layers. Many axons appeared swollen with irregular outline and revealed disorganization of their neurofilaments and many swollen mitochondria with destroyed cristae (Figs. 5 d, 5 e). Many processes of astrocytes related to blood capillaries were swollen and markedly enlarged with swollen mitochondria (Fig. 5 f).

Examination of ultrathin sections obtained from animals treated with Cd and AA at the same time for two months revealed that most of nerve cells had euchromatic nuclei, normal mitochondria, few dilated rER in-between normal ones (Fig. 6a) while few of them still having irregular nuclear envelopes (Fig. 6b) with peripheral condensation of their chromatin (Fig. 6c). Processes of nerve cells showed regular arrangement of their neurofilaments and the myelinated fibers showed regular arrangement of their myelin (Fig. 6d). The blood capillaries were surrounded with moderately enlarged processes of astrocytes (Fig. 6e).

3.3. Morphometric and statistical Results

A significant increase ($P < 0.05$) in the number of vacuolated cells with dark nuclei was found in group III compared to control, group II and group IV. In addition, a significant

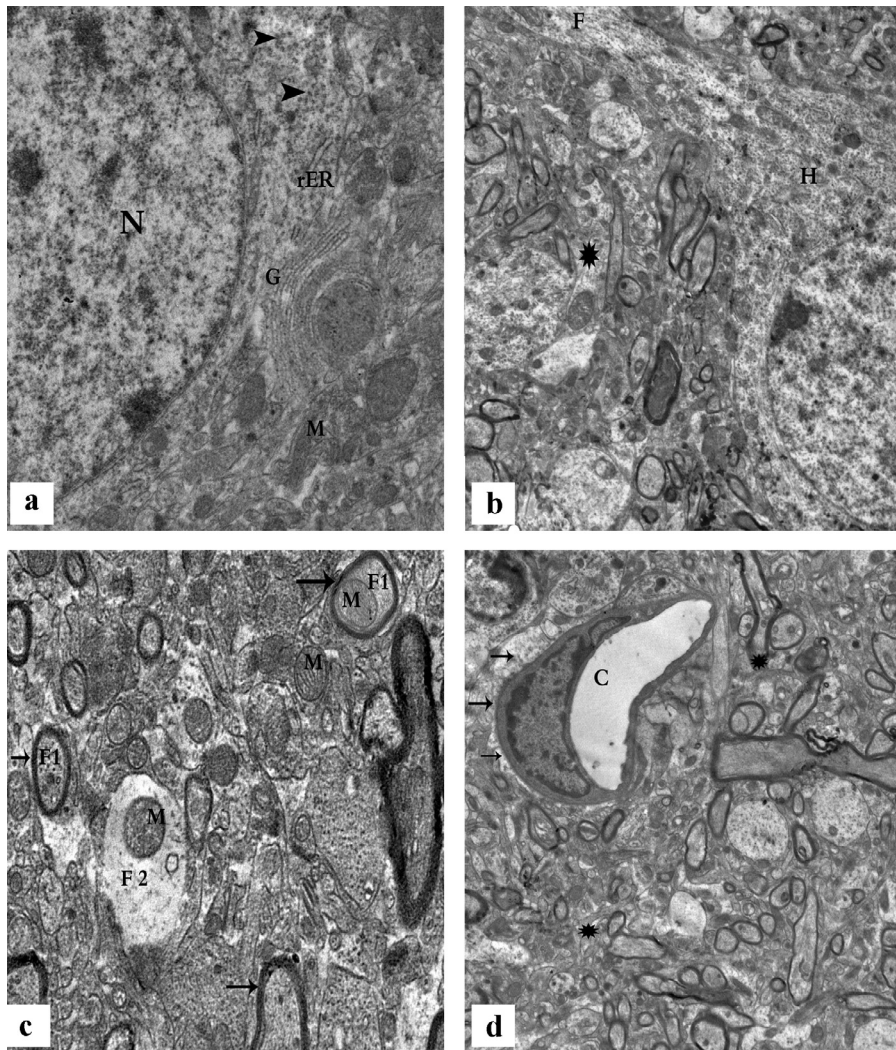


Fig. 4. Electron micrographs of a section in the cerebral cortex of adult control rat; (4a): showing a perikaryon of normal nerve cell containing euchromatic nucleus with normal nuclear envelope (N), normal cytoplasm containing (rER), polyribosomes (arrow head), Golgi (G) and mitochondria with normal density (M) (4000 X). (4b): showing the axon hillock (H) and initial part of the axon with regular distribution of neurofilaments (F). Notice the normal surrounding neuropil (*) (1500 X). (4c): showing multiple nerve fibers either myelinated with regular myelin sheath (F1) or unmyelinated (F2), where numerous neurofilaments, microtubules and mitochondria (M) are seen. Notice the regular arrangement of myelin sheaths (arrow) (4000 X). (4d): showing a blood capillary (C) surrounded by many processes of astrocytes (arrow). Notice the normal surrounding neuropil (*) (1500 X).

increase was found in group IV compared to group I and II and a significant decrease in the same group comparing to group III (Table 1, Histogram 1).

4. Discussion

Cadmium (Cd) is an inorganic environmental toxicant of increasing importance due to industrialization, occupational contaminant, smoking, and lack of effective therapy for cadmium poisoning. Cd toxicity may impact human health through the persistent exposure to small doses over a long period of time and its exposure can produce both acute and chronic tissue injury. The effect of cadmium on the various regions of the central nervous system has been conventionally described by many authors who confirmed that the brain is a major cadmium depot in the body and

has no known mechanism for its elimination [19]. So, the cerebral cortex was chosen as a target in this work to study the histological changes after Cd exposure and the possible protective role of ascorbic acid on these changes.

In the present work, examination of sections of cerebral cortex, by light and electron microscope, did not demonstrate substantial differences among rats in control and ascorbic acid-treated groups and it was similar to the normal ultra structural picture. On the other hand, Cd administration induced histological changes in the all layers of the cortex involved different types of cells especially pyramidal and granule cells and some neuroglia. These results were previously recorded by many authors who established that Cd produced neuropathological and neurochemical alterations in the brain causing severe damage including encephalopathy, peripheral neuropathy and

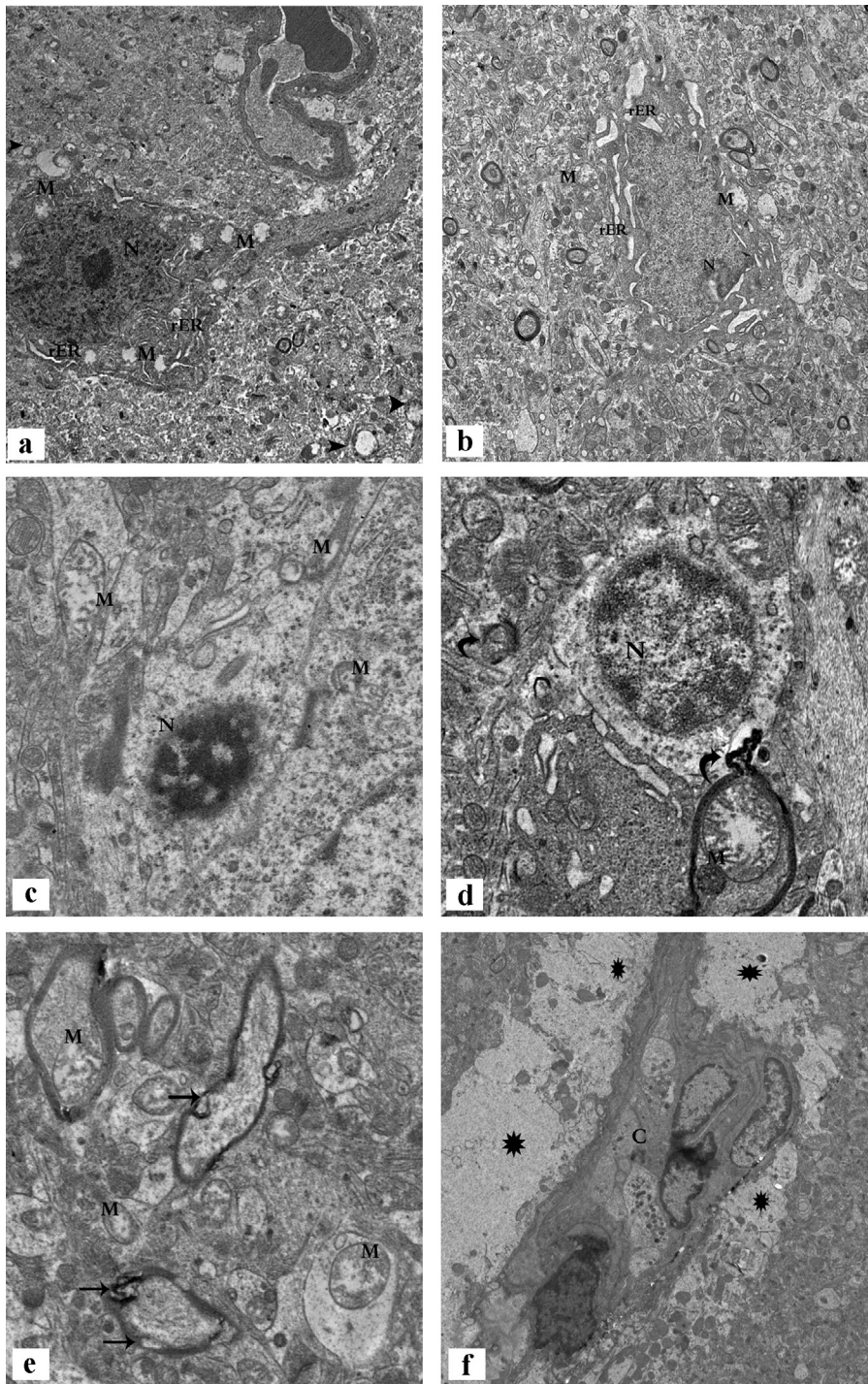


Fig. 5. Electron micrographs of a section in the cerebral cortex of Cd treated rat; (5a): showing cytoplasm of nerve cell with irregular nuclei (N), swollen mitochondria with partial loss of their cristae (M), dilated (rER). Notice the vacuolated mitochondria in the neuropil (arrow head) (1500 X). (5b): showing a nerve cell with irregular nucleus (N), dilated (rER) and swollen mitochondrion (M) with partial loss of its cristae (M) (1500 X). (5c): showing nerve cell with irregular condensed chromatin (N) surrounded with swollen mitochondria (M) (4000 X). (5d): showing small nerve cell with peripheral condensation of their nuclear chromatin (N) surrounding with nerve fibers with irregular and discontinuous myelin sheath (curved arrow) and swollen mitochondria with partial loss of cristae (M) (4000 X). (5e): showing neuropil with many nerve fibers having splitting and irregular arrangement of their myelin (arrow). Notice the swollen mitochondria with partial loss of their cristae (M) (4000 X). (5f): showing blood capillary (C) surrounded with markedly enlarged processes of astrocytes (*) (1500 X).

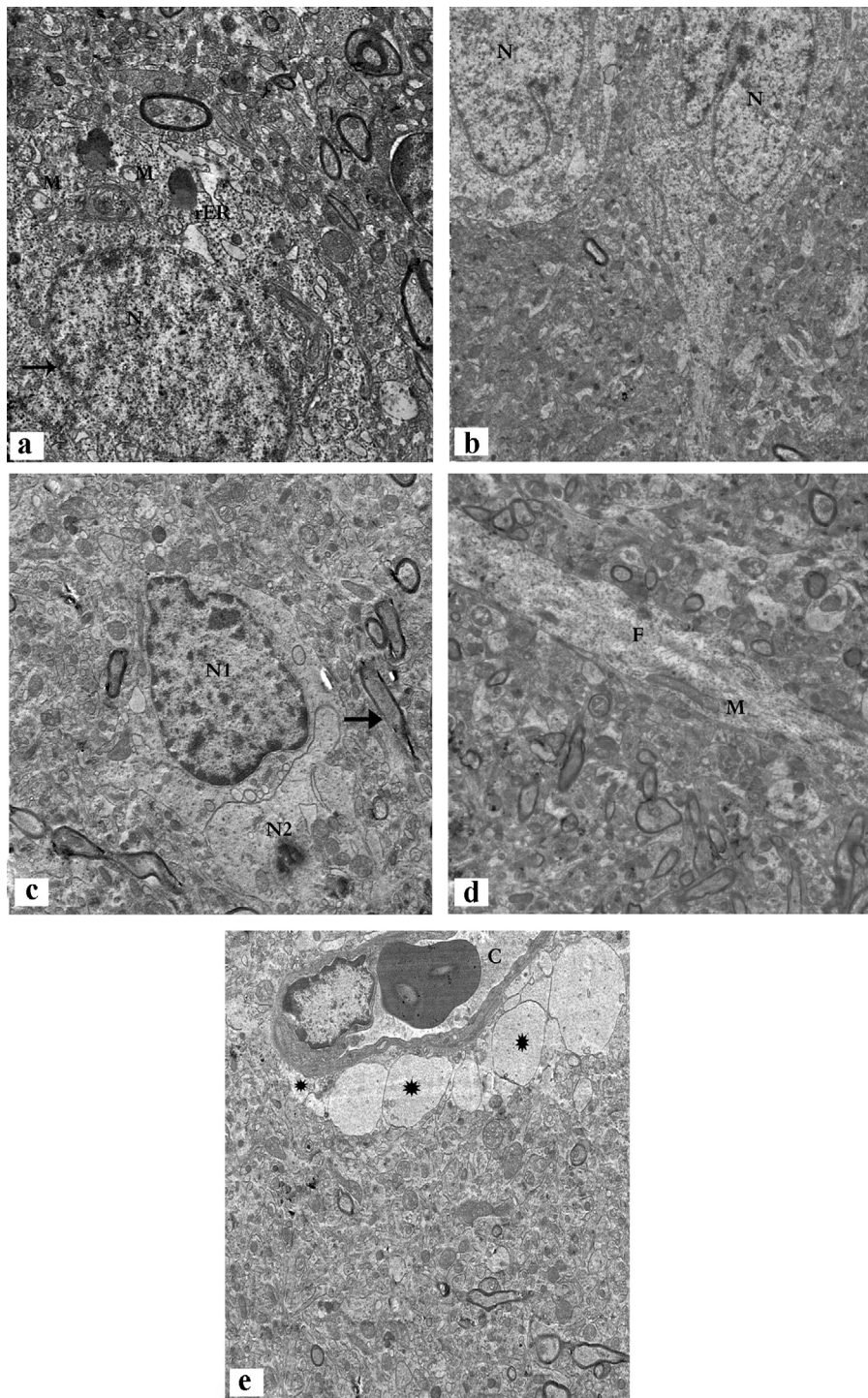


Fig. 6. Electron micrographs of a section in the cerebral cortex of Cd and AA treated rat; (6a): showing a nerve cell with regular euchromtic nucleus (N), regular nuclear envelope (arrow), mild dilated (rER) and swollen mitochondria with partial loss of cristae (M) (2000 X). (6b): showing irregular nuclei (N), with normal euochromatin and more or less normal organelles. (1500 X). (6c): showing irregular nuclei with slight peripheral condensed chromatin (N1). Notice a nerve cell with condensed nucleus (N2), nerve fibers with irregular myelin sheath (arrow) (1500X). (6d): showing part of an axon with regular arrangement of neurofilaments (F) and normal mitochondria (M) (1500X). (6e): showing blood capillary (C) surrounded with moderately enlarged processes of astrocytes (*).

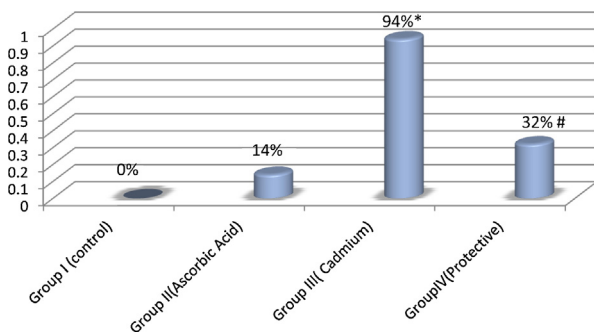
Table 1

Percent of vacuolated cells with dark nuclei in control and experimental groups.

Groups	Number of vacuolated cells with dark nuclei
Control (group1)	0%
Group II (Ascorbic acid)	14%
Group III (Cadmium)	94%*
Group IV(Protective)	32%#

* Significant compared to control and groups II and IV.

Significant increase compared to control and group II& significant decrease compared to group



Histogram 1. Percent of vacuolated cells with dark nuclei in control and experimental groups. (1500X).

hemorrhage [20]. In addition, others found that this heavy metal could affect the brain parenchyma and neurons, leading to lower attention, hypernociception, olfactory dysfunction and memory deficits [21].

The pathophysiologic mechanisms underlying Cd neurotoxicity remain not completely understood and may be due to many theories. Cd ion affects the structure of nucleic acids, the activity of certain enzymes, the uptake of some catecholamines and the levels of various neurotransmitters. It also blocks the synaptic transmission at peripheral cholinergic and adrenergic synapses [22]. In addition, Cd ions can produce tissue-injury by reactive oxygen species (ROS) generation with depletion of antioxidant defense system causing an imbalance between pro oxidants and antioxidants. ROS can attack the polyunsaturated fatty acid in the biomembrane and induce free radical chain reactions [23]. Specifically, Cd decreased the total antioxidant status resulting in a decline in glutathione content, superoxide dismutase and glutathione S-transferase activity, inducing lipid peroxidation and oxidative stress. The brain tissues are highly susceptible to oxidative damage due to its high utilization of oxygen and its poorly developed antioxidant defense mechanism. In addition, Cd directly destroyed the choroid plexus as it was accumulated and sequestered to protect cerebral cortex from the fluxes of Cd in blood [24].

Cd also stimulates ROS production due to an inhibitory effect on mitochondrial electron transport. ROS may lead to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems [25].

The appearance of dark neurons which is seen in this work reflects a certain phase of apoptosis as they displayed markedly condensed cytoplasm and nucleoplasm

[26]. Other authors believed that dark small neurons are usually ischemic due to substantial abnormalities in the capillary wall with subsequent disorders in the structural elements of the blood-brain barrier [27]. Lastly, the distorted shrunken cells seemed to be a result of damage structural and functional biosynthesis of cell proteins; nucleic acids, certain enzymes and various neurotransmitters [22].

The irregular outline and loss of the shape in most of pyramidal cells in this work could be correlated with the cytoskeletal disorganization noticed by electron microscope. These affected cells revealed major ultrastructural changes in most organelles indicating cell degeneration. The increase of cytosolic calcium and alteration in mitochondrial permeability next to oxidative stress can cause mitochondrial damage. The mitochondrial damage is reflected on its function leading to rapid degeneration of the cells [23]. The mitochondrial and nuclear disorders were considered to be secondary to direct toxicity on neuronal cells that induced disorder of biochemical events. The rER dilatation may be due to lipid peroxidation [28].

Regarding the cytoplasmic vacuolation in the nerve cell of the Cd treated rats; it was a result of lipid peroxidation theory, in addition to damage of the cell membrane as well as membranes of other organelles. Such damage is specifically followed by an increase in the sodium permeability which exceeds the capacity of pump to extrude the sodium. Accumulation of sodium in the cell leads to an increase in water content in the cell leading to its swelling [29]. The increased vacuolation was confirmed by image and statistical analyses.

The degenerative changes in the axons of nerve cells were in agreement with Rai et al [30] who found similar toxic effects induced by cadmium in the myelinated nerve fibers of cerebral cortex, optic nerve and retina, and also with Gerspacher et al., who found damage to the cytoskeleton in Cd treated cells [31].

The axons changes were recorded as a component of a dying-back process of neuronal injury while the myelination's disruption was attributed to the changes in myelin basic protein secondary to membrane damage and axonal degeneration. Demyelination (loss of the myelin sheath) can occur as a response to axonal degeneration or secondary to the oxidative stress. Dysmyelination (alterations in the myelin sheath), was attributed to increased water content in degenerating nerve causing intramyelinic edema and oedematous splitting at various levels of the myelin lamella [32]. In addition, the free radicals potentially damage the oligodendrocytes [responsible for myelination) and neurons causing cell membrane damage and impairment of myelination [33].

The vacuolation in the surrounding neuropil might be attributed to the shrinkage of cells and withdrawal of their processes secondary to cytoskeletal affection leaving pericellular spaces. They are indicative of neuronal death and are consistent with neuronal necrosis as seen in early stages of ischemic, hypoxic/ischemic, hypoglycemic and excitotoxic states [34]. The neuropil vacuoles represented the swollen neuronal processes and presynaptic nerve endings, while the cytoplasmic vacuoles corresponded with swollen mitochondria [35].

Regarding the neuroglia, the enlarged processes of astrocytes may be as a result of lipid peroxidation theory and an increase in the sodium permeability resulting in sodium accumulation in the cell followed by increase in water content and swelling of the cell [29,36]. The astrocytes were considered the primary target for cadmium-intoxication even before any neuronal affection. These cells played a major role in neurotransmitter uptake and metabolism, neurotransmitter receptor expression, neurotrophic factor-secretion and secretion of extracellular matrix protein [36].

In the present work, examination of specimens obtained from animals treated with cadmium concomitantly with ascorbic acid (AA) showed improvement in the nerve cells and hardly ever the affected cells are noticed. These findings were in agreement with other study who found that the ascorbic acid prevented the free radicals caused oxidative damage of the cell membrane [37] and also protects the neurons against exitotoxic cell death [38]. It was suggested that vitamin C exerts neuroprotective action through scavenging the oxygen free radicals [39]. Raghu et al added that a decrease in lipid peroxidation, an increase catalase activities were the reason for AA neuroprotection [40].

Most of nerve cells, their axons and myelin sheath and neuroglia are more or less as in control group. These agreed with several animal studies on Cd toxicity which found that AA supplementation decreased SOD activity, increased lipid peroxidation, apoptosis, and necrosis, through scavenging the ROS generated by Cd administration [41]. This was probably related to its antioxidative properties, and affection on Cd absorption and distribution [42].

The brain is a susceptible tissue to oxidative stress due to high level of free radicals, and high amounts of unsaturated fatty acids. The free radical oxygen has potential role in neural cell damage. Exogenous antioxidant such as ascorbic acid (vitamin C), α -tocopherol (vitamin E), and β -carotene can be effective on neuronal cell protection due to the effect of ROS on neuronal cell damages and fast consumption of endogenous scavenging antioxidants [43].

This improvement of the histological changes induced by Cd is also coincided with the previous electron microscopy and electrophysiological studies which 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 [44]. In addition, ascorbic acid plays prophylactic effects on Cd-induced organ toxicity via enhancing cadmium transport and decrease its uptake in rat intestinal segments [45].

Additionally, ascorbic acid showed antiapoptotic effect through decreasing Bax protein, enhancing Bcl-2 protein [46], and exerted neuroprotective action through decreasing lipid peroxidation and increasing catalase activities [47].

So, cellular damage caused by Cd exposure can be prevented by free radical scavengers or antioxidants, which further strengthens the hypothesis that free radicals play a key role in Cd toxicity [48].

In conclusion, ascorbic acid administration exhibited an ameliorating effect on the Cadmium induced cerebral cortical neurotoxicity in an experimental model of adult male

albino rats. So, it is recommended that awareness should be focused on AA and antioxidants supplement; particularly in areas where Cd contamination is expected, as important protective measure for the neurotoxicity. Considering the mild degenerative changes still observed in some cells in this work, further intensive experimental and clinical study may be required to adjust the dose and time of AA treatment.

Conflicts of interest

There are no conflicts of interest.

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