Study on Toxic Effects of Lead Acetate on Cerebellar Cortical Tissue of Adult Albino Rats and the Role of Vitamin E as a Protective Agent.

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Abstract

Lead is a common industrial poison that persists in the environment and has many toxic effects on different organs & tissues especially on the central nervous system. This study aimed to investigate the effects of lead administration on cerebellar cortex of adult male albino rats and the possible protecting effects of vitamin E. Materials and Methods: Forty male albino rats, (160 ± 10 g weight) were divided into four groups, ten rats each. In the control group rats were received distilled water daily, 2nd group rats were given 30 mg/ kg lead acetate dissolved in distilled water orally daily for two months, 3rd group rats were pretreated by 100 mg/ kg vit. E orally 6 hours before administration of lead acetate by the same dose as group 2 for two months, the 4th group rats were given the same dose of lead acetate then withdrawal was done for one month. Tissue specimens then prepared for light and electron microscopic examinations. The results: By light microscope, in rats treated with lead, the number of Purkinje cells showed a significant decrease in comparison to control group, and appeared shrunken, distorted in shape with irregular nuclei, while in rats treated with lead and vitamin E, there was marked improvement of these alterations. Electron microscopic examination showed Purkinje cells with ill defined nucleus, vaculated or rarified cytoplasm and small electron dense mitochondria. Granular cells showed vaculated cytoplasm and mitochondria with destroyed cristae. In case of vitamin E administration, marked protection against these changes was observed, while the withdrawal group showed very little or no improvement.

Keywords

Lead acetate, cerebellum, vit. E, protective, withdrawal, ultrastructure.

Introduction

Lead (Pb) is a highly toxic heavy metal that persists in the environment and the human body and can disrupt neurological & other biological body functions (Bauchi et al., 2016). Chronic poisoning by it is one of the major public health hazards especially in developing countries (Flora et al., 2012).

Small amount of lead is excreted in urine and the rest accumulates in various body tissues, mainly the CNS which may result in structural changes that can persist even after lowering of its blood level (Sidhu and Nehru, 2004; Taib et al., 2004; Flora et al., 2006; Ibrahim et al., 2012).

Lead was reported to produce oxidative stress by generating release of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, hydrogen peroxide and lipid peroxides which increase oxidative damage of cellular materials (Ercal et al., 2001 and El-Nekeety et al., 2009).

Depending on the observation that free radicals were generated during the pathogenesis processes induced by lead, it was presumed that supplementation of antioxidants will interrupt or minimize the damaging effects of lead and improve the effects of chelating agents (Flora et al., 2003).

Vitamin E is a lipid soluble membrane-bound antioxidant which protects cell membrane against oxidative stress (Soylu et al., 2006), and has powerful neuro-protective effects (Crouzin et al. 2010).

Aim of work

To investigate the toxic effects of lead on cerebellar cortex of albino rats and to evaluate the possible protecting role of vit. E.

Materials and methods

-Animals

Forty adult male albino rats weighing 160 ±10 g. were used. Rats were obtained from the Animal House of Assiut University, Egypt. Animals were housed in standard conditions and fed on normal diet and water ad libitum.

Rats were divided into four groups as the following:

Group I (control): 10 male albino rats had received 2 ml distilled water by oro-gastric tube daily for 2 months.
Group II (treated): 10 male albino rats had received lead acetate in a dose of 30 mg/ kg which equals 1/20th of LD50 according to (Sujatha et al., 2011), dissolved in 2 ml distilled water by oro-gastric tube daily for 2 months.

Group III (Lead + vit. E): 10 male albino rats had received 100 mg/ kg vit. E according to (Bashandy 2006) orally 6 hours before administration of lead acetate by the same dose as group II for two months

Group IV (withdrawal): 10 male albino rats were given the same dose of lead acetate like group II then withdrawal was done for one month.

Chemicals: Lead acetate was purchased from Hemajet company, Egypt and Vitamin E capsules were obtained from PHARCO pharmaceuticals

Methods

*Light microscopic examination: After the animals were sacrificed, the skull was opened and the two cerebellar hemispheres were removed then fixed in 10% formalin. After the fixation the samples were dehydrated in a graded series of ethanol, and embedded into paraffin. Blocks of samples were sectioned on a microtome (5-7 um) thick sections and stained with haematoxylin and eosin (H&E) according to Bancroft and Gamble (2007).

* Semithin & Electron microscopic examination: 4 rats from each group were used, they were perfused intracardially with 4% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 hours and then fixed in 1% osmium tetroxide in phosphate buffer for 2 hours. Semithin sections (0.5-1um) were prepared using ultramicrotome and stained with toluidine blue according to Gupta et al. (2007) then examined by light microscope

Ultrathin sections (50-80 nm) from selected areas of trimmed blocks were made and collected on copper grids. The ultrathin sections were then stained with uranyl acetate & lead citrate for 10 minutes according to (Hayat 2000), and examined by transmission electron microscope (Jeol EM) in the unit of electron microscope, Faculty of Medicine, Assiut university.

Morphometric study

The number of Purkinje cells per field was counted in the 4 animal groups under the study through using a computer assisted image analyzer in the histology department, faculty of medicine, Assiut University. Measurement was done viewing semithin sections by x100 objective lens in five non overlapping fields in ten randomly chosen sections from four different animals for each group. The data then analyzed using statistical package for the social science (SPSS, version 22).

Results

Table 1 shows that the number of Purkinje cells in the group of animals treated with Pb and in the withdrawal group was significantly lowered in comparison to control. While the group treated with Pb and vitamin E showed no statistical difference in comparison to control.

Light & electron microscopic results

Group I (Control):

A) By light microscope: H&E and toluidine blue stained sections showed normal histological structure in the form of outer molecular layer (ML) which is mainly formed from fibers with few small stellate cells and basket cells. Middle Purkinje cells in the Purkinje layer (PL) are arranged in one row of large pyriform or flask shape cells, with clear nuclei, prominent nucleoli and cytoplasm. The inner most layer contain the granular cells (GL) which are closely packed rounded small cells

B) By electron microscope: the cerebellar cortex showed Purkinje cell body with well defined nucleus with electron dense nucleolus, numerous mitochondria, free ribosomes, Golgi bodies and strands of rough endoplasmic reticulum (RER).

Group II (treated with lead):

A) By light microscope: by H&E stain the Purkinje layer showed shrinkage of the Purkinje cells (empty spaces around them) with increased acidophilia, while toluidine blue stained sections showed distorted shape Purkinje cells and their nuclei appear irregular.

B) By electron microscope: the Purkinje cells appear with irregular ill-defined nucleus with increased condensation of nuclear chromatin and indentations of the nuclear envelope. The cytoplasm is rarified with small dense mitochondria and the cells are surrounded by empty spaces. Granular cells show increased condensation of nuclear chromatin inside their nuclei. The nuclei are surrounded by a shell of vacuolated cytoplasm, the mitochondria appear with destroyed cristae.

Group III (treated with Lead + vit. E): A) By light microscope: H&E stained sections showed almost normal appearance of (ML) (PL) (GL). Toluidine blue stained sections showed Purkinje cells with vesicular nucleus and prominent nucleolus

B) By electron microscope: the Purkinje cells appeared with euchromatic nucleus and prominent electron-dense nucleolus. The cytoplasm showed cisternae of rough endoplasmic reticulum (RER) around the nucleus, and some dilated cisternae of perinuclear Golgi. Granular cells appeared with rounded heterochromatic nuclei, surrounded by a shell of cytoplasm containing free ribosomes, strands of rough endoplasmic reticulum (RER), and mitochondria.

Group IV (withdrawal):

A) By light microscope: H&E stained sections showed irregular Purkinje cells with darkly stained nuclei & cytoplasm , while the molecular and the granular layers are apparently normal. Toluidine blue stained sections show Purkinje cells which appear irregular in size and shape with darkly stained nuclei and cytoplasm. The molecular layer revealed perineuronal spaces around stellate cells and basket cells while the granular layer was apparently normal.

B) By electron microscope: ultra-structure examination revealed Purkinje cells with euchromatic nuclei which shows heterochromatin. The cytoplasm shows free ribosomes, strands of rough endoplasmic reticulum , free lysosomes, areas of vacuolated cytoplasm, and mitochondria with destroyed cristae.
Table (1): comparison between number of Purkinje cells in the control group of animals and each of the other four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD</th>
<th>P-value</th>
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<tbody>
<tr>
<td>I (Control)</td>
<td>8.23 ±1.87</td>
<td>0.001*</td>
</tr>
<tr>
<td>II (Treated)</td>
<td>5.93 ±1.57</td>
<td>0.078</td>
</tr>
<tr>
<td>III (Lead +vit.E)</td>
<td>7.42 ±1.73</td>
<td>0.031*</td>
</tr>
<tr>
<td>IV (withdrawal)</td>
<td>6.36 ±1.65</td>
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</table>

*Student-t test where P<0.05 = significant value

Fig 1: A photomicrograph of a section in the cerebellar cortex of a rat from the control group showing a molecular layer (M) formed of few small stellate cells and basket cells. Purkinje cells (P) in the Purkinje cell layer (PL) are arranged in one row of large pyriform somata with prominent nucleoli and cytoplasm along the outer margin of the granular layer (G), whereas the granular layer shows tightly packed small rounded cells. H&E X 1000

Fig 2: A photomicrograph of a section in the cerebellar cortex of a control rat showing the molecular layer (M), the Purkinje cell layer (PL), and the granular layer (G). Purkinje cells (P) are arranged in one row of large pyriform somata with clear vesicular nuclei and prominent nucleoli along the outer margin of the granular layer. Toluidine blue X 1000

Fig 3: An electron micrograph in the cerebellar cortex of control rat showing; part of Purkinje cell body. Well defined euchromatic nucleus (N) with electron dense nucleolus is seen; numerous mitochondria (m), free ribosomes (r), Golgi bodies (Gol) and strands of rough endoplasmic reticulum (rER) can be observed. Notice: The nuclear cap region (arrow head). (X 5000).

Fig 4: A photomicrograph of a section in the cerebellar cortex of a Lead treated group showing part of the PL with shrinkage of the Purkinje cells (P). Their nuclei appear irregular. Notice the increased acidophilia (arrow) (H&E x 1000).
Fig 5: A photomicrograph of a semithin section in the cerebellar cortex of a lead-treated adult albino rat showing Purkinje cells (P) with distorted shape and irregular nucleus. Notice the empty spaces around them. (arrow) (Toluidine blue x 1000).

Fig 6: An electron micrograph of ultrathin section in the cerebellar cortex of a rat treated with Pb showing Purkinje cell that has an irregular euchromatic nucleus (N) with increased condensation of nuclear chromatin and indentations of the nuclear envelope. The cytoplasm is rarified with small dense mitochondria. The cell is surrounded by empty spaces (star) X 7200.

Fig 7: An electron micrograph of an ultrathin section in the cerebellar cortex of a lead-treated rat showing granular cells (GC) with increased condensation of nuclear chromatin (arrows) inside their nuclei (N). The nuclei are surrounded by a shell of cytoplasm showing free ribosomes (r), mitochondria (M) with destroyed cristae, and areas of vacuolated cytoplasm (v). × 4800.

Fig 8: A photomicrograph of a section in the cerebellar cortex of a rat treated by both lead and Vit E. showing almost normal appearance of Purkinje cells (arrow) in the Purkinje cell layer (PL), (M) layer and the granular (G) layer show an almost normal appearance. H&E, × 1000.
Fig 9: A photomicrograph of a section in the cerebellar cortex of a rat treated by both lead and Vit E. showing Purkinje cells with a vesicular nucleus and a prominent nucleolus (arrow) in the Purkinje cell layer (PL). Note the molecular (M) and granular (G) layers. Toluidine blue, × 1000.

Fig 10: An electron micrograph of an ultrathin section in the cerebellar cortex of a rat treated by both lead and Vit E. showing a Purkinje cell (PC) with a euchromatic nucleus (N) and a part of a prominent electron-dense nucleolus (arrow head). The cytoplasm shows cisternae of rough endoplasmic reticulum (rER) around the nucleus, and some dilated cisternae of perinuclear Golgi (G). x7200

Fig 11: An electron micrograph of an ultrathin section in the cerebellar cortex of a rat treated by both lead and Vit E. showing granular cell layer (GC) with their rounded hetero-chromatic nuclei (N) surrounded by cytoplasm which shows free ribosomes (r), rough endoplasmic reticulum (rER), and mitochondria (M).x 4800

Fig 12: A photomicrograph of a section in the cerebellar cortex of the withdrawal group showing some Purkinje cells (arrows) that appear irregular and darkly stained. The molecular layer (M) and The granular layer (G) are apparently normal. H&E, × 1000.
Fig 13: A photomicrograph of a section in the cerebellar cortex of the withdrawal group showing Purkinje cells (arrows) that appear irregular in size and shape with darkly stained nuclei and cytoplasm. The molecular layer (M) shows perineuronal spaces. The granular layer (G) is apparently normal. Toluidine blue × 1000.

Fig 14: An electron micrograph of an ultrathin section in the cerebellar cortex of a rat of the withdrawal group showing a Purkinje cell (PC) with a normal nucleus (N) that shows heterochromatin (arrow). The cytoplasm shows free ribosomes (r), strands of rough endoplasmic reticulum (rER), lysosomes (Ly), and mitochondria (M) with damaged cristae. X 4800

Fig 15: An electron micrograph of an ultrathin section in the cerebellar cortex of a rat of the withdrawal group showing granule cells (GC) with rounded heterochromatic nuclei (N) showing increased condensation of nuclear chromatin (arrow). The nuclei are surrounded by a shell of cytoplasm containing free ribosomes (r), areas of vacuolated cytoplasm (v), and mitochondria (M) with destroyed cristae. X 4800

Discussion
The neurotoxic effects of high level lead exposure has been proved in both animals and human, where the damage involves peripheral and central nervous systems (Brenet, 2006). Many factors account for the neurotoxic effects of lead; they include integrity of blood-brain barrier, lead-binding proteins, cellular scavengers (e.g., glutathione) and interactions with other micro-nutrients (Sidhu and Nehru, 2004)

Vitamin E is the most important lipophilic antioxidant which stays mainly in the mitochondria thus helping to maintain membrane stability. Also, it decreased the cell death which is due to free radicals. The ingestion of vitamin E gives a protection against lipid peroxidation through its anti-oxidant action (Serbecic and Beutelspacher, 2005)

In the present study, lead induced damage and disorganization in Purkinje cells was evident. By
light microscope they were shrunken with distorted shape and their nuclei appear irregular. Ultrastructural examination proved the Purkinje cells damage in the form of irregular euchromatic nucleus, rarified cytoplasm, the mitochondria are small dense with destroyed or dilated cristae and the cells are surrounded by empty spaces. Alterations of Granular cells in the form of increased condensation of nuclear chromatin, the nuclei are surrounded by a shell of vacuolated cytoplasm, and the mitochondria appeared with destroyed cristae were also detected. These findings are in agreement with (Engin Deveci, 2006), who reported that when rats received lead acetate in their drinking water for 60 days degeneration in the neuron cells was evident. The histological findings were also similar to those reported by Villeda Hernandez et al., (2006), Macauley et al., (2008), Amal and Mona (2009), Sohair el al. (2010), Musa et al. (2012) and Fakunle et al. (2013). Changes in neuronal cells observed in this study could be explained by the generation of reactive oxygen species, and depletion of antioxidant reserves. Lead exposure inactivates glutathione molecule (important endogenous antioxidant) by binding of its sulfhydryl group directly (Pajović et al., 2003) and (Sanders et al., 2009). Zhu et al. (2006) and Mattson et al. (2008) reported that Mitochondrial dysfunction and distortion have been recorded in many neurodegenerative diseases which associated with oxidative damage.

On the other hand, the animals treated with Pb and vitamin E revealed marked improvement in the altered histological architecture of cerebellar cortex, whereby light microscope the purkinje, molecular and the granular cell layers appeared almost normal, while by electron microscope the purkinje cells appeared with euchromatic nucleus, normal prominent nucleolus, strands of RER in the cytoplasm and some dilated Golgi cisternae around nucleus. Nearly similar findings were reported by Amal and Mona (2009) who recorded that the co-treatment of rats by Pb and antox (mixture of vitamins E, C, A and selenium) prevented most of the histopathological distortion. The mild histological alteration in cerebellar cortex of rats treated by Pb and vitamin E when compared with the marked distortion in rats treated with Pb alone is a powerful indicative on the anti-oxidant and the neuro-protective effects of vitamin E. Our results coincide with Crouzin et al. (2010) who stated that the pretreatment of hippocampal neurons of rats by vitamin E will give a long lasting protection against oxidative damage induced by Fe ++ ions by preventing Ca** entry to neuronal cells. Also Alzoubi et al. (2012) reported that vitamin E is a strong antioxidant which has a neuro-protective effect on the brain against cognitive impairment which resulted from chronic sleep deprivation. The findings of this study are in line with Rao and Sharma (2001) who mentioned that vitamin E has a protective antioxidant effect against mercury induced toxicity on male reproductive system, while Gulec et al. (2006) reported that vitamin E give a protection against oxidative damage induced by formaldehyde on liver tissue and plasma of rats. Bashandy (2006) concluded that the co-administration of both vitamin E and C protects the liver against lead induced lipid peroxidation.

Regarding the withdrawal group of rats in which cessation of exposure was done for one month after 2 months treatment by lead, the ultrastructure examination revealed partial recovery of purkinje cells and granular cells, but most of purkinje cells have permanent Pb induced alterations. The persistence of degenerative changes in purkinje cells after Pb withdrawal can be explained by the specific kinetics, where fraction of lead after absorption is redistributed and stored in bone (Timchalk et al., 2006), then slowly released from bone and redistributed to soft tissues including cerebellar tissue (Kosnett, 2001). These results are in line with Sohair el al. (2010) who found that withdrawal of Pb resulted in minimal regression in the structural alterations of cerebellar cortex.

**Conclusion**

Administration of Pb to the studied animals has led to morphological alteration in the neuronal cells in the cerebellar cortex, while the co-administration of Pb and vitamin E resulted in amelioration of these effects to a great extent due to its anti-oxidant activity.

**Recommendations**

We recommend more investigations on the neurotoxic effects of lead on laboratory animals and human, with regular administration of vitamin E where lead exposure could not be avoided.

**References**


دراسة على تأثيرات السامة لخلايا الرصاص على نسيج قشرة المخيخ في الجرذان البيضاء البالغة ودور فيتامين E كعامل وقائي

سميرة محمد صالح 1 و فاطمة ياسين عبد المجيلى 2

الرصاص هو مادة صناعية شائعة في البيئة ولها العديد من التأثيرات السامة على أعضاء الجسم والأنسجة المختلفة خاصة على الجهاز العصبي المركزي.

الهدف من اجراء البحث: أجريت هذه الدراسة لفحص تأثيرات تناول خلايا الرصاص على نسيج قشرة المخيخ للجرذان البيضاء من الذكور البالغين وتأثيرات الحماية المحتملة لفيتامين E.

الطريقة البحث: تم تقسيم أربعة عشر فئران لكل منها (150± 10) جم إلى أربعة مجموعات، عشة فئران لكل منها. في المجموعة الأولى (الضابطة) تم إعطاء الفئران الماء المقطر يوميا، بينما أعطيت فئران المجموعة الثانية 30 ملجم / كجم من خلايا الرصاص مذابة في الماء المقطر عن طريق الفم يوميا لمدة شهرين، وتم معالجة فئران المجموعة الثالثة بواسطة 100 ملجم / كجم من فيتامين E بالفم 6 ساعات قبل إعطاء خلايا الرصاص نفسها جرعة المجموعة الثالثة لمدة شهرين، أعطيت المجموعة الرابعة من الفئران نفس الجرعة من خلايا الرصاص ثم إيقاف الرصاص لمدة شهر واحد، ثم أعدت عينات الأنسجة المفحوصة المجهرية الضوئي والإلكتروني.

النتائج: بالفحص المجهرية الضوئية في الفئران التي عولجت بالرصاص، أظهر عدد الخلايا بيكتريوسيكوب الضوئي في الفئران التي عولجت بالرصاص، أظهر عدد خلايا بيكتريوسيكوب الضوئي قل في الفئران المعالجة بالرصاص و فيتامين E. كان هناك تحسن ملحوظ في هذه التغيرات، و بالفحص المجهرية الضوئية، و في حالة إعطاء فيتامين E، لوحظت حماية واضحة من هذه التغيرات، في حين أظهرت مجموعة الانتصاب تحصينا ضئيلا جدا أو منعدما.

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