

Toxicopathological effects of lead acetate on the brain of male mice

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Summary

The aim of the present study is to determine the influence of different doses of lead acetate on the central nerves system. 25 Swiss strain white mice were used, each weighing about 30 – 32 g, divided into three groups, first group (n=10) treated with 0.5 ml. contain 150 mg/kg/body weight lead acetate via stomach tube daily for 40 days, second group (n=10) treated with 0.5 ml. contain 300 mg/kg/body weight lead acetate via stomach tube daily for 40 days. While the third group (n=5) served as control and were given mammalian physiological saline. Clinical signs were reported during the course of the study, then at day 40 post treatment , all animal were sacrificed and post mortem examination was done and any gross lesions were reported, then the pieces of brain was fixed in 10% formalin for 72 hours .

The pathological results showed congestion of cerebellum and cerebrum of both treated group but the 2nd group was more sensitive. Histopathological examination of 1st group expressed moderate pathological lesions, characterized by extracellular edema around neuron cells and Virchow Robbin space , as well as proliferation of astrocytes in the white matter , also central chromatolysis of neurons and Nissle granules with homogenous pink their cytoplasm in 1st G. while in 2nd G the main lesions characterized by severe congestion of blood vessels with inflammatory cells infiltration in the lumen of arachnoids' space and brain parenchyma as well as hemorrhage with aggregation of microglia in the wall of blood vessels which characterized by round shape and clear cytoplasm (microgliosis). Also severe neuron degeneration, with Alzheimer's type-II astrocytes are reported in other animals characterized by pairs observed surrounded by clear space. And there is no clear pathological lesion in control group.

In conclusion the present study investigated that the lead acetate affected on the brain tissue, and the degree of influence depended on the concentration of the toxic dose.

Key word :- Toxicopathological effects of lead , Lead acetate, Brain, Central chromatolysis, Al-Zheimer's type-2

التأثير المرضي السمي لخلات الرصاص في دماغ ذكور الفئران البيضاء

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الخلاصة

أجريت هذه الدراسة لبيان تأثير الجرعة السمية لمحلول خلات الرصاص من خلال دراسة التغيرات المرضية العيانية و المجهرية على الجهاز العصبي المركزي لذكور الفئران البيضاء تجريبياً. استخدم في الدراسة 25 من ذكور الفئران البيضاء، تراوحت اوزانها بين 30-32 غم و اعمارها بين 6-8 اسابيع حيث قسمت الى ثلاث مجاميع، جرعت المجموعة الأولى بـ(0.5 مل يحتوي على 150 ملغم/كغم من خلات الرصاص) بينما، جرعت المجموعة الثانية بـ(0.5 مل يحتوي على 300 ملغم/كغم من خلات الرصاص) يومياً ولمدة 40 يوم بواسطة التجريب الفموي، بينما أعطيت المجموعة الثالثة المحلول الفيزيولوجي واعتبرت مجموعة السيطرة. تم ملاحظة العلامات السريرية طيلة فترة التجربة وبعد إجراء الصفة التشريحية، أظهر الفحص المجهرى وجود الخبز والاحتقان مع التغيرات التنكسية والنخرية في عصبونات المخ والمخيخ، وافات التلين البؤري في جذع الدماغ والمخ، أما في حيوانات المجموعة الثانية أظهر الفحص المجهرى انحلال في المادة الكروماتية لخلايا العصبونية والتي تميزت بفقدانها النواة مع وجود حبيبات نسل المتميزة يلونها الأرجواني داخل سايتوبلازم الخلية العصبية، ووجود التنكس الشديد المتمثل بوجود أزواج خلايا الزهايمر من النوع الثاني. نستنتج من هذه الدراسة بأن هنالك تلف شديد في دماغ الفئران الناتج عن التسمم بخلات الرصاص، والذي يزداد تأثيره بزيادة الجرعة السمية المتناولة.

Introduction

Lead was conceded as a bluish-gray, solid metal, heavy, low melting, and dense, with chemical formula is Pb (comes from the Latin word "Plumbum") (1).

Pb have three oxidation states Pb(O), the metal Pb(II), and Pb(IV) and four isotopes: ^{208}Pb (51-53 %), ^{206}Pb (23.5-27 %), ^{207}Pb (20.5-23 %), and ^{204}Pb (1.35-1.5 %). Lead isotopes are the stable decay product of three naturally radioactive elements: ^{206}Pb from uranium, ^{207}Pb from actinium, and ^{208}Pb from thorium and molecular weight 207.20, melting point 327.4 °C, boiling point 1,740 °C, density at 20 °C is 11.34 g/cm³, and it is insoluble in water (2).

It should be noted that the prevalence of lead poisoning, at least that caused by drinking water containing lead, in ancient Rome is far less than what is traditionally believed (3). Today, most exposure in developed countries is the result of occupational hazards, leaded paint and leaded gasoline, which continuous to be phased out in most countries (4).

Lead acetate were used in dyeing of textiles, waterproofing, varnishes, lead driers, chrome pigments, gold cyanidation process, insecticide, anti-fouling paints, analytical reagent and hair dye (5).

Neurotoxicity of lead acetate was acknowledged in human and animal species, in acute encephalopathy, most serious consequences of plumbs, since permanent impairment of the central nervous system, and may occur particularly in young children (6). About 25% of children affected with lead encephalopathy may suffer permanent damage to the central nervous system. This damage is usually reflected in behavioral and educational abnormalities with or without accompanying mental retardation, and accumulated within the nucleus of neuron and renal cells in vivo and in vitro (7) and in liver cells in vitro, that cause direct DNA damage and mutation (8). The brain is the most sensitive target of lead toxicosis because it contains relatively low levels of enzymes that are capable of protecting it against oxidative stress (9).

So the goals of the present study are to determine the influence of different doses of lead acetate on the brain.

Materials and Methods

Twenty five, Swiss strain white mice, with ages about 8 – 12 weeks and body weight ranged between (30 – 35g) were obtained from animal house of the pathology department at college of veterinary medicine, Baghdad University. Animals were randomly divided into three treated groups. 1st group (n=10 treated with 0.5 ml. contain 150 mg/kg/body weight Lead acetate, via stomach tube daily for 40 day), 2nd group (n=10 treated with 0.5 ml. contain 150 mg/kg/body weight Lead acetate, via stomach tube daily for 40 day). While the 3rd group (n=5) served as control and were given mammalian physiological saline. During the course of study, any clinical signs were reported, then at day 40 post treated, all animal were sacrificed and post mortem examination was done and any gross lesion were reported, than the brain specimens were fixed in 10% formalin for 72hour and processed according to (10) and the histopathological changes were observed under light microscope.

Results

In 3rd group, no clear pathological lesions (Fig. 1 & 2). The pathological lesions in 150 mg/kg characterized by severe proliferation of astrocyt in white matter of cerebellum form nodular lesion called astrogliosis (Fig.3), congestion of blood vessels with neutrophils infiltration in the lumen were seen in piameter (Fig.4), in other section extracellular edema was reported, characterized by dilated of Virchow-Robbin space around blood vessels and glial cells (Fig.5), as well as congestion of B.Vs in the choroid plexuses was reported, together with increase of astrocytes and oligodendrocytes (Fig.6), edema in granular layer also observed. The present study revealed a central chromatolysis of neurons characterized by the neurons become rounded or enlargement (Fig.7), lost of nuclei and Nissle substances with

homogenous pink their cytoplasm, also in other section cerebral vascular prominent due to enlargement of endothelial cells with astrocytosis that characterized by swelling and proliferation of astrocyte in white matter (Fig.8).

Histopathological examination in 300 mg/kg showed severe congestion of B.Vs with inflammatory cells infiltration in their lumina of piamatter (Fig.9), also there is hemorrhage in the brain tissue, as well as congestion of B.Vs in brain parenchyma with aggregation of microglia in wall of B.Vs (Fig.10), characterized by rod shape nuclei without, clear cytoplasm (microgliosis) characterized by aggregation of microglia cells, with elongated nuclei in the cerebellum (Fig.11). Also our histopathological study revealed to active microglia aggregation around necrotic space in molecular region (Fig.12). On other hand there is congestion of B.Vs in arachnoid space with neutrophils infiltration in their lumen, astrocytes aggregation around B.Vs also reported (Fig.13). In other section mullierain degeneration also present which characterized by elongated , irregular space surrounded by microglia cell, together with severe neurons degeneration (center chromatolysis) which characterized by eosinophilic cytoplasm , rounded border, loss or a central nuclei with lost of Nissle granules(Fig.14). The detection of brain tissue , indicated that *Alzheimer type-II* astrocytes were reported which characterized by pair of nuclei arrangement surrounded by clear space(Fig.15), with severe congestion of B.Vs in brain parenchyma, coincident with present of extracellular edema characterized by rounded space around neuron and dilated Virchow-Robbin space (Fig.16).

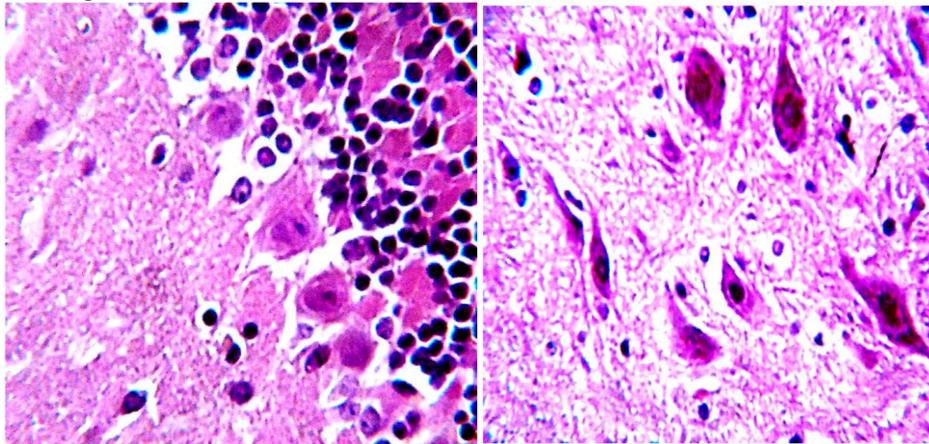


Fig:1, Histopathological section Brain control with normal structure of (H&E stain. 40X)

Fig:2, Histopathological section Brain control with show normal structure of perivascular space

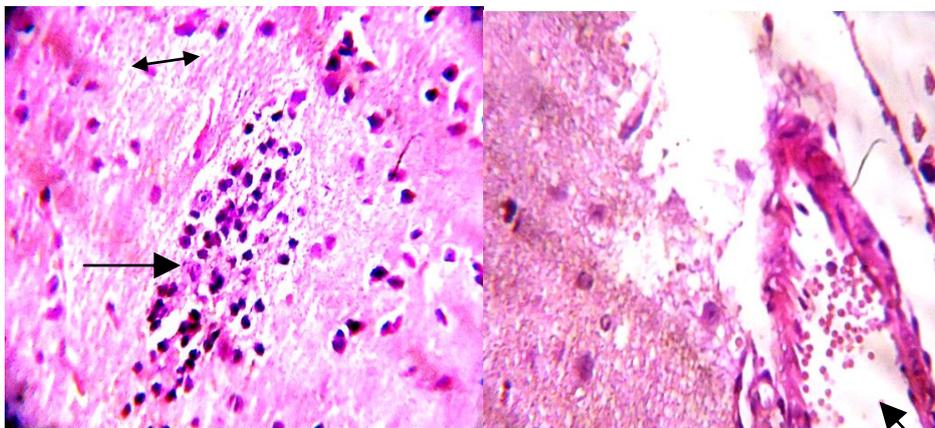


Fig.3, Histopathological section in Brain of T1 shows proliferation of astrocyte in white matter of cerebellum and aggregation of neutrophils (H&E stain. 40 X)

Fig.4, Histopathological section in Brain of T1, show congestion of B.V with neutrophils in the lumen around congested B.Vs(H&E stain. 40 X)

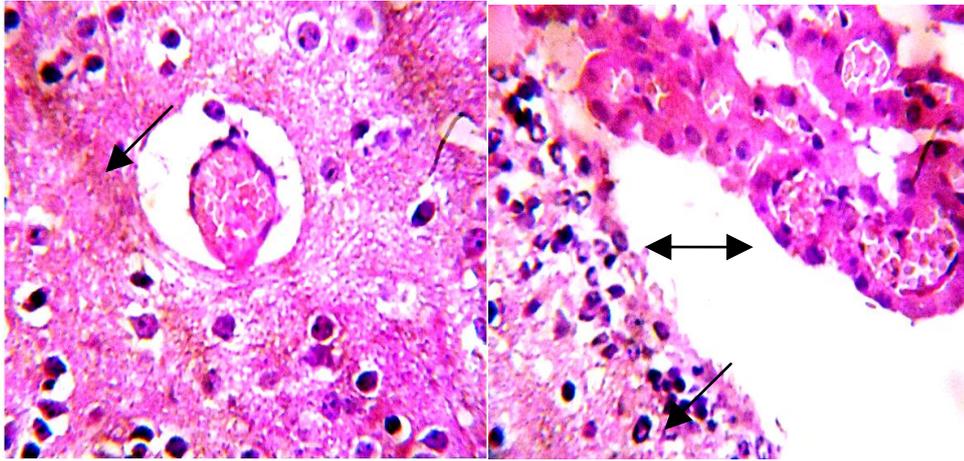


Fig:5 Histopathological section in Brain of T1, extracellular edema characterized by Virchow-Robin space(→) (H&E stain. 40 X)

Fig:6 Histopathological section in Brain of T1, show show increase of astrocyte and oligodendrocyte, dilation of with edema in granular layer(→) and congestion of B.Vs in choroid plexis (↔) (H&E stain. 40 X)

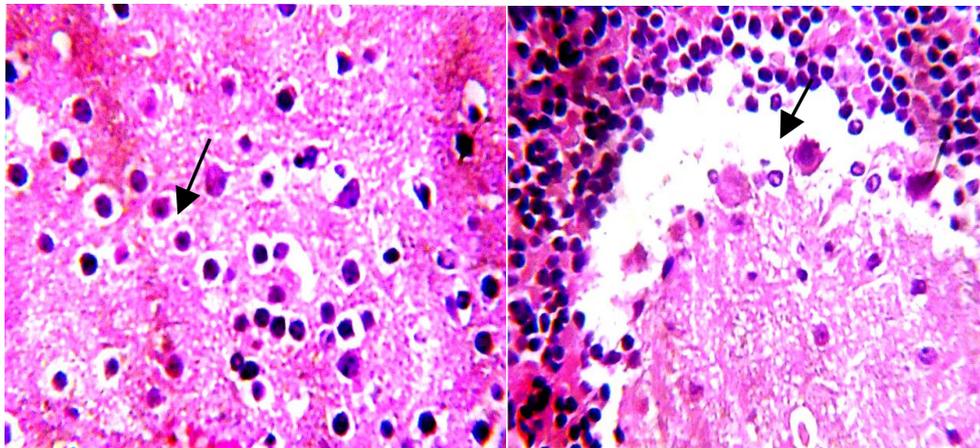


Fig:7 Histopathological section in Brain of T1, show per neuronal edema in granular layer → (H&E stain. 40 X)

Fig:8, Histopathological section in Brain,T1, show center chromatolysis of neurons characterized by rounded or enlarged neurons.→ (H&E stain.40X)

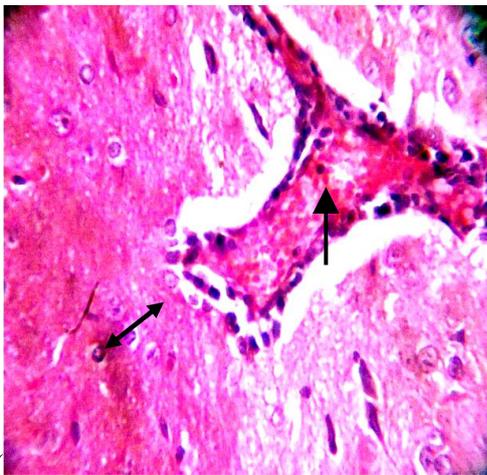


Fig:9, Histopathological section in Brain,T1, show cerebral vascular prominent duct → and mononuclear cells aggregation around B.Vs ↔

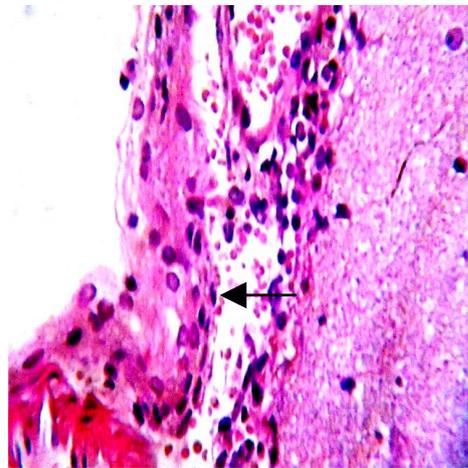


Fig:10, Histopathological section in Brain,T1, show severe congestion of B.V with inflammatory cells in the lumen → (H&E stain. 40 X) (H&Estain. 40 X)

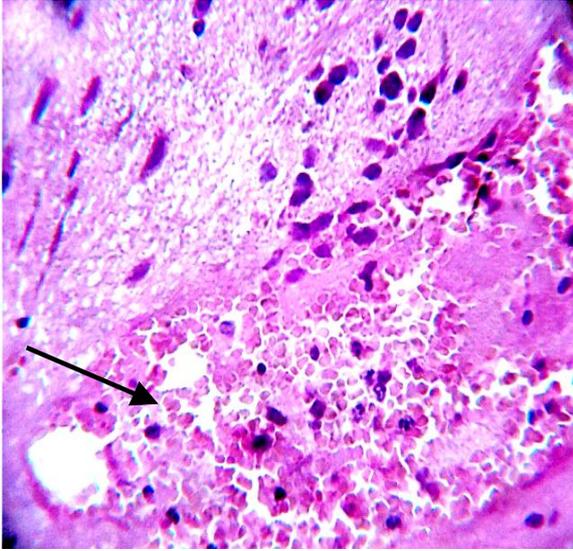


Fig:11, Histopathological section in Brain, T2, show hemorrhage in the brain tissue with aggregation of microglia → (H & E stain. 40 X)

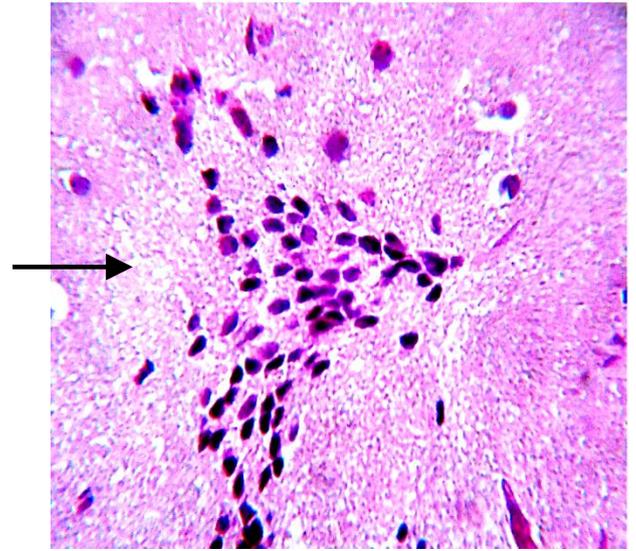


Fig:12, Brain, T2, show microgliosis characterized by aggregation of microglia cell with elongated nuclei in the cerebellum → (H & E stain stain. 40 X)

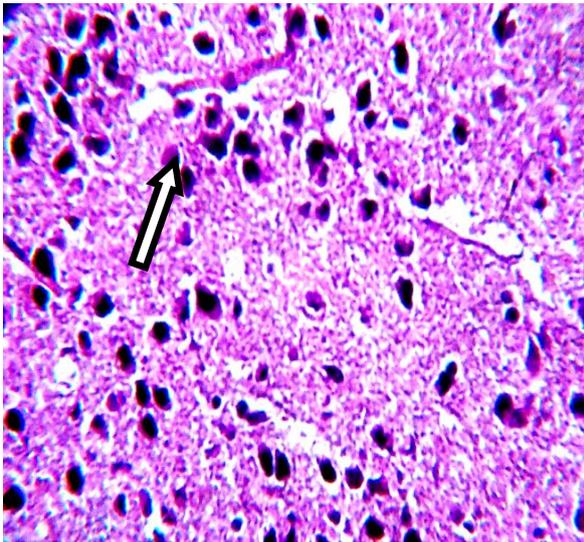


Fig:13, Histopathological section in Brain, T2, show focal astrocytes aggregation around congestion B.V (H & E stain. 40 X) →

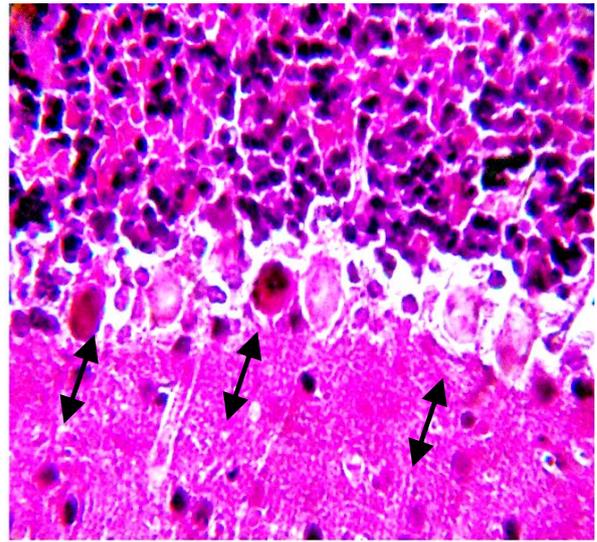


Fig:14 Histopathological section in Brain, T2, show central chromatolysis with severe neurons (H& E stain. 40 X) ←→ degeneration, eosinophilic cytoplasm rounded border, loss of a central nuclei with lost of Nissle granules ←→ (H & E stain. 40 X)

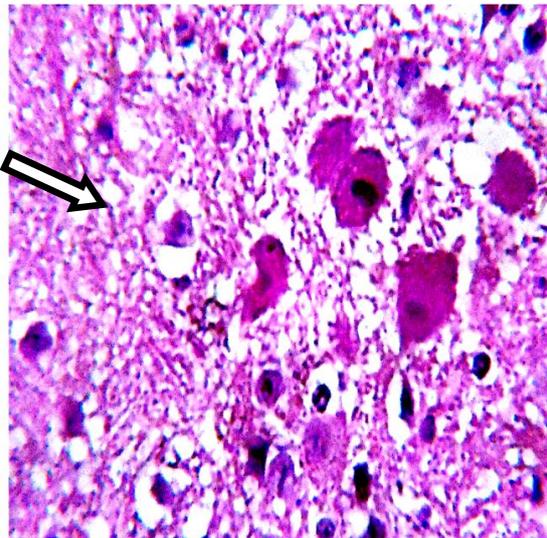
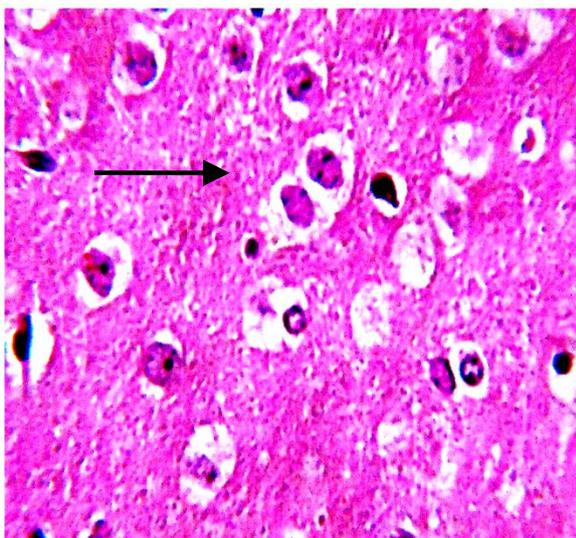


Fig:15 Histopathological section in Brain,T2,show *I I* astrocyte characterized by pair of nuclei arrangement surrounded by clear space → (H&E stain. 40X)

Fig:16 Histopathological section Brain,T2,show *type-II* astrocyte characterized by space of extracellular edema characterized by space around neuron → (H&E stain. 40X)

Discussion

The results showed severe damage and destruction to the brain tissue parenchyma, oedematous brain, and present edema may be due to severe inflammation that present in brain, with damage in the B.Vs and disturbances in blood brain barrier and basement membrane and pericytes through the space between neighboring endotheliocytes. Leading to escape of fluids from B.V to the nervous tissues. That lead to increase the pressure on the brain tissue causes irreversible tissue and brain damage. This result agreed with (11) who reported that oedematous fluid seen grossly in spinal cord, brain stem, cerebrum, and cerebellum, with present perineuronal and perivascular odema microscopically in rat after treated with 75 mg/kg. B.W/day of lead acetate for 60 days.

The degenerative changes and necrosis, occur because neuronal mitochondrial activity is affected by lead, with disruptive effects on synaptic transmission in the brain, and lead picked up by mitochondria and produced swelling and distortion of mitochondrial cristae, uncoupled energy metabolism, inhibited cellular respiration, and altered calcium kinetics follow, the organelles mediating cellular energy metabolism (12) . The results were in agreement with (13) who investigated that the neurotoxic effect of lead on the anterior horn cells was due to the effect of lead on glucose metabolism.

Present of Demyelination in axon because of Phigh myelin-associated content of brain, which makes it vulnerable to the propagation of peroxidative events. Lead exposure may be causes reduction in the accumulation of brain myelin in the developing brain , also it effect on glucose metabolism (14). This evidence was in consistence with (15) who predicted that if Schwann cell damage were to be implicated in lead neuropathy, demyelinated internodes would be distributed randomly among myelinated fibers in the affected nerves, whereas segments demyelinated in a process secondary to axonal degeneration would tend to be clustered within certain fibers.

Lead also interferes with excitatory neurotransmission by glutamate, which is the transmitter at more than half of synapses in the brain and glutamate receptor thought to be associated with neuronal development and plasticity in the N-methyl-D-aspartae (NMDA) receptor, which is blocked selectively by lead . This idea is in consistence with idea mentioned by(12) .

The observation of *Alzheimer type-II astrocyte* in this study was in acceptance with those previously reported by several investigators (16) explained that increase activity of reactive oxygen species (ROS), it binds to sulphydral groups and interferes with many sulphydral-containing enzymes, which replace Zinc in some enzymes, that interference with the action of γ -amino butyric

acid (GABA), as well as obstruction cholinergic function in association with reduced extracellular calcium. That effect on dopamine uptake by synaptosomes, and it may be interference with calcium needed to activate protein kinase C in brain capillaries.

The present study investigated that the lead acetate affected on the brain tissue, and the degree of influence depended on the concentration of the toxic dose.

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