



Cytogenetic Effect of Heavy Metal Exposure in Alzheimer's Disease Rat Model

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Abstract: The widespread heavy metal, potassium dichromate (PDC), presents an actual threat because of its high toxicity to all body organs. This research was designed to study the possible cytotoxicity and genotoxicity exerted by PDC on Alzheimer's disease (AD) and the prospect negative effect on AD induced in rats. The current research was performed on forty male rats, classified similarly into 4 sets: normal control, AD control was done by intraperitoneal injection of lipopolysaccharide (LPS) at a dose 250 µg/kg body weight for 7 days, AD rats intraperitoneally injected once by PDC 10 mg/kg body weight, and PDC-intoxicated rats (same dose as the third group). The fourth group served to compare the effect of PDC alone. Twenty-four hours after the PDC injection, rats were sacrificed to collect their brain hippocampi (CA3 region) to study its histopathological structure, acetylcholinesterase (AChE) gene expression by RT-PCR, malondialdehyde (MDA) content and catalase (CAT) activity spectrophotometrically. Additionally, cells of the bone marrow were harvested from the rat femoral bone to undergo *in-vivo* micronucleus (MN) assay. The outcomes entailed that AD rats with PDC significantly altered the hippocampal histology, vanished neuronal survival count, increased AChE gene expression, MDA content and CAT activity in AD rats more than normal and PDC-only rats. Furthermore, PDC significantly elevated frequency of MN in AD rats' bone marrow cells. In conclusion, the study results evidenced that PDC exerted a tremendous harm to the AD case, evident by all previously mentioned outcomes.

Keywords: Potassium Dichromate, Alzheimer's Disease, Lipopolysaccharide, Micronucleus, Cytotoxicity, Genotoxicity.

1. INTRODUCTION

Genotoxic heavy metals are compounds that encounter an ability to enter the brain and accumulate, which in several cases done by ruining the blood brain barrier. These pernicious compounds accumulate and trigger pathways that alter neurochemistry which supports intellectual function ¹. Chromium (Cr) is a heavy metal found naturally and represents the seventh most available element on earth. There are two oxidation conditions that chromium occurs in the natural environment, Cr³⁺ [Cr(III)] and Cr⁶⁺ [Cr(VI)] ². Out of the two

chromium forms, Cr⁶⁺ is the most labile, mobile and toxic form. Potassium dichromate (PDC) (K₂Cr₂O₇) is a great source of Cr⁶⁺, where it is used in the chrome industry to manufacture pigments and dyes, in addition to antiseptics preparation ³. The widespread use of Cr⁶⁺ to produce stainless steel leads to occupational exposure of millions of workers all over the world to the hazardous vapour during its production ⁴. PDC is a potent oxidizer, so it is harmful to biological systems. Due to its solubility, it is highly transportable in the milieu and offers an excessive source of toxicity, mutagenicity, teratogenicity, and carcinogenicity. It can create reactive oxygen species throughout its reduction process, and these suspicious compounds can result

in damage to the DNA, lipids and proteins of the cell⁵.

One of the major neurodegenerative disorders worldwide is Alzheimer's disease (AD), that is characterized by continuing decrease of cognitive jobs and memory. Based on recent statistics, about 6.2 million Americans aged 65 and older are living with Alzheimer's dementia in 2021⁶. Its pathological changes are observed mainly in the hippocampal and cortical regions⁷. AD is developed due to decrease in acetylcholine (ACh) in the brain by extensive breakdown via acetylcholinesterase (AChE), deposition of amyloid- β ($A\beta$) extracellularly and hyper-phosphorylated tau intracellularly, forming neurofibrillary tangles⁸. ACh plays important roles in learning and memory⁹. The cholinergic system is rigorously affected in AD, implicating its role in memory. A common feature in AD brain is the presence of AChE in high levels, which is commonly associated with β -amyloid plaques and neurofibrillary tangles¹⁰.

Neuro-inflammation is known to have a role in the pathological processes of AD¹¹. Activation of microglia may contribute to the neurodegenerative process through the discharge of proinflammatory cytokines, and other toxic yields¹². Lipopolysaccharide (LPS) is a powerful endotoxin, derived from Gram-negative bacterial cell wall, that can start different pathological impacts in humans and animals. Latest studies suggest that chronic exposure to LPS can result in neurodegeneration and consequent AD¹³.

Several studies have shown that imbalance in metal homeostasis may lead to neuronal loss in neurodegenerative diseases¹⁴. Maynard suggested that dietary metals could influence $A\beta$ deposition in the brain under certain conditions, through decreasing $A\beta$ clearance¹⁵. Moreover, it was observed that acetylcholine level was decreased in cortex, hippocampus and amygdala of rats intoxicated with heavy metals, clarifying that cholinergic insufficiencies can cause cognitive impairment¹⁶.

The micronucleus (MN) test is the utmost commonly undertaken *in vivo* mammalian genotoxicity assessment. It was known in the 19th century end¹⁷. This assay was established as a technique to estimate the frequency of micronucleated erythrocytes amongst normal erythrocytes, which have no nuclei throughout hematopoiesis. The sample used in this assay is either bone marrow or peripheral blood cells of animals^{18,19}. Formerly, it was stated that MN occurs when acentric fragments, or the whole chromosomes, cannot integrate into the daughter nuclei during mitosis. It has been revealed that mitotic apparatus (spindle, kinetochores) disorders and diminished role of topoisomerase II is captivated in micronucleus

formation. Accordingly, counting MN in cytoplasm of the interphase daughter cell has been utilized to evaluate aneugenic or clastogenic chromosomal DNA damage²⁰.

Based upon the above-mentioned data, our study aimed to elucidate the PDC potential on AD in rats, by investigating the cytotoxicity and genotoxicity of PDC following an intraperitoneal injection. In addition, studying changes in the expression of AChE, oxidative stress biomarkers levels, as well as histopathological impact on the hippocampus responsible for memory.

2. METHODS

2.1. Reagents and Chemicals:

Lipopolysaccharide from Escherichia coli; O127:B8 was obtained from Sigma Aldrich, Saint Louis, MO, USA. Potassium dichromate was purchased as an orange powder from Alpha Chemika, India. RNA extraction QIAamp RNeasy Mini kit, purification of Total RNA kit and SYBR Green Master Mix for PCR were bought from Qiagen, Germany. Primers used were supplied from Metabion, Germany. Hematoxylin, eosin and toluidine stains were supplied from Merck, Germany. The other used chemicals were analytically highly pure.

2.2. Animals:

Forty male Wistar rats, aged 8-12 weeks old, and weighed about 180-220 g were obtained from the animal house colony of The Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt), to be utilized in the current study. The rats were tail-marked to be distinguished, individually weighed and randomly allocated to one of the four groups to avoid significant differences within body weights and age of each group. Rats were accommodated in common polypropylene cages in the animal house located in Faculty of Pharmacy, October 6 University, three to four rats per cage, at room temperature (25 ± 2 °C), under controlled light (10h light: 14h dark). Animals were permitted to adapt to their milieu for one week preceding the experimental procedures. Normal pellet diet and H₂O *ad libitum* were delivered to the rats along the experiment period.

2.3. Experimental design:

The rats were allocated into 4 groups (10 rats in each group). The first group was named as normal control, received normal saline i.p. for 7 days. The second group was considered as the AD control, received LPS 250 μ g/kg body weight dissolved in cold 0.9% NaCl, i.p. for 7 successive days²¹, to

induce systemic inflammatory response and consequent neuroinflammation similar to AD. The third group was allocated for AD rats receiving the heavy metal, PDC, where AD was induced just as the second group, followed by PDC (10 mg/kg body weight single i.p. injection freshly dissolved in saline²²) one hour after the last LPS injection. While the fourth group was assigned to investigate the effect of PDC alone, as they were PDC-injected just as the third group, but one hour after the last i.p. saline injection that lasted for 7 successive days. The overall design is shortly summarized in Figure 1.

All rats were then ether- euthanized, and sacrificed by decapitation after 24 hours of their last injection. Brains of 6 rats/ group were harvested instantly after decapitation and rinsed with cold normal saline, hippocampi were dissected out and quickly saved at -80°C for determination of AChE gene expression, MDA content and CAT activity. We used 10% formaldehyde (buffered) to fix the brains of 4 rats from each group for subsequent histopathological examination. Moreover, both of the femurs were removed from 4 rats/ group, and were allocated for micronucleus assay.

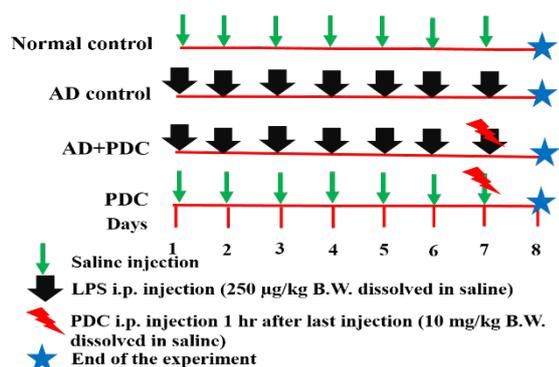


Figure 1: Diagram presenting the experimental design

2.4. Brain hippocampal assessment of AChE genetic expression:

This was undergone using quantitative real time polymerase chain reaction (qRT-PCR) technique. Concisely, isolation of RNA was done via RNeasy RNA abstraction kit, according to the producer's directions (Qiagen). DNase I was added to RNA and reverse transcription following manufacturer's protocol, was done. Quantitative real-time PCR was performed with SYBR Green master mix in a Stratagene MX3005P real time PCR machine, with the subsequent cycling circumstances: preliminary denaturation at 95 °C for 7 min, then 40 cycles at 95 °C for 10 seconds and 60 °C for 30 seconds. Sequences for primers utilized were: AChE (ahead: 5'-GAAGCAGGAATGACGAAGCAG-3') and (backward: 5'-GAGGAGGAAAAGGAAGAAGAG-3')²³, in

addition to β-actin as housekeeping gene (forward: 5'-TCCTCCTGAGCGCAAGTACTCT-3') and (reverse: 5'-GCTCAGTAACAGTCCGCCTAGA-3')²⁴. The mRNA was standardized to β-actin mRNA, and comparative gene expression was computed by the stratagene MX3005P software via the relative CT means (2^{-ΔΔCT})²⁵.

2.5. Brain hippocampal assessment of malondialdehyde content:

Malondialdehyde content was measured in accordance with the manufacturer's instructions (Biodiagnostics, Egypt) as mentioned by Ohkawa *et al.*²⁶. Briefly, aliquot from hippocampal tissue homogenates was mixed with thiobarbituric acid to react with malondialdehyde at acidic pH for 30 min at temperature of 95°C, to produce thiobarbituric acid reactive (TBAR) product. The absorbance of the pink product was read at 534 nm.

2.6. Brain hippocampal assessment of catalase activity:

Catalase activity was measured in accordance with the manufacturer's instructions (Biodiagnostics, Egypt) as mentioned by Aebi *et al.*²⁷. Briefly, catalase in hippocampal tissue homogenate reacted with a known quantity of H₂O₂. This was followed by adding catalase inhibitor after exactly one minute to halt the reaction. In the existence of horse radish peroxidase, the residual H₂O₂ reacted with 3,5-Dichloro-2-hydroxybenzene sulfonic acid, together with 4-aminophenazone to produce a colored compound, whose color intensity is contrariwise the quantity of catalase in the sample, then absorbances were read at 510 nm.

2.7. Bone marrow assessment of micronucleus:

Micronucleus test was carried out on 4 rats per each group^{28,29}. Bone marrow was prepared as follows. Both femurs were detached, and all the adhering muscles were dissected out. The bone marrow was removed out from both femurs by flushing with 1 ml fetal calf serum then subsequently centrifuged at 1200 rpm for 15 min. This was followed by supernatant aspiration and cells fixation by cold 3:1 methanol: acetic acid. Afterwards, two smears per animal were set by adding drops of the pellet on slides at angle equals to 45°, then left to dry for 20, then stained by May-Grun Wald and Giemsa stains. Three types of cells were counted under the microscope, nucleated cells (N) these represents the white blood cells, polychromatic erythrocytes (PE) and PE with micronucleus (MN-PE). The item of recording was the micronucleated PE, not the micronucleus itself; therefore, if a cell had more than one micronucleus, it was recorded as one MN-PE. All slides were examined under light microscope

(Leica DM2500) at magnification power 100X, using emersion oil for cell counting. Two variables were scored in this analysis:

The cytotoxicity, was expressed as the number of PE to total number of PE and N (%):

$$\text{Cytotoxicity \%} = \frac{\text{PE} \times 100}{\text{PE} + \text{N}}$$

And the genotoxicity, was expressed as the number of MN-PE to total number of PE (%):

$$\text{Genotoxicity \%} = \frac{\text{MN-PE}}{\text{PE}} \times 100$$

2.8. Brain hippocampal histopathological examinations:

The formaldehyde-preserved brain samples were fixed in paraffin wax to be sectioned into 4 μm thick sagittal brain sections from the CA3 hippocampal region by means of rotatory microtome. Then the samples were truncated, dehydrated in ethanol, cleared by dipping in Xylene, and then stained by using either Hematoxylin and Eosin (H&E) or using Nissl staining (Toluidine blue stain) for revelation of intact and degenerated neurons. Nissl stained tissues were examined by summing up of intact neurons by means of microscopic camera (Full HD), managed via Leica application unit for analysis of tissues (Leica Microsystems, Wetzlar, Germany).

2.9. Analysis of statistics:

Data were demonstrated as mean ± S. E.M. Statistical significance was calculated as P < 0.001 for AChE gene expression, MDA level, catalase activity and Nissl staining neuronal counting. Concerning the micronucleus test, significance was taken as P < 0.05. All statistics was carried out using one-way analysis of variance (ANOVA), then Tukey's multiple comparison test, and were accomplished with GraphPad Prism 5.1 software (GraphPad Software, La Jolla, CA, USA).

3. RESULTS

3.1. Alterations in hippocampal AD biomarker:

As depicted from figure (2), AD control group resulted in significant increase in AChE gene expression by 5.8 folds when compared to normal group. The effect of PDC injection to AD-rats was much harmful, reaching 7.6 folds when compared with normal rats and 1.3 folds of the AD control. Moreover, PDC single injection resulted in 2.5 folds'

elevation when compared to normal rats. Furthermore, when PDC was injected to AD rats it resulted in 3.04 folds' significant increase than PDC alone.

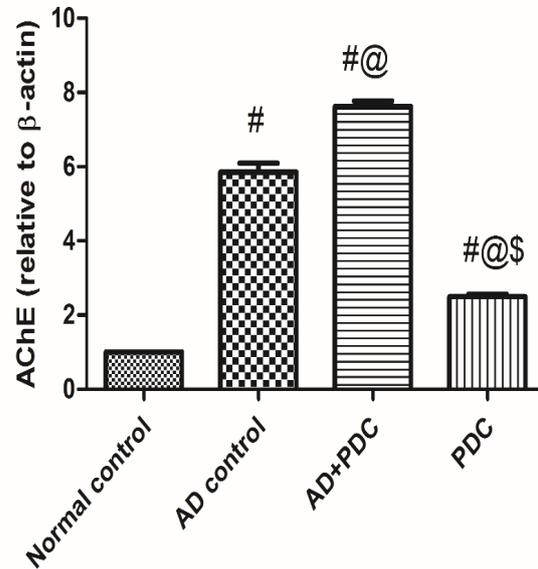


Figure 2: Changes in hippocampal AChE gene expression. Data represent means ± S.E.M. (n=6). AChE gene expression in brain hippocampal tissues from rats treated with either saline (normal control), LPS (AD control) or LPS+PDC was determined using qRT-PCR. Statistical analysis was carried out using one-way ANOVA followed by Tukey's multiple comparison test. As compared with normal control (#), AD control (@) and AD injected with PDC (\$), relative to β-actin. All groups were compared at p < 0.001. AChE: acetylcholinesterase, AD: Alzheimer's disease, PDC: potassium dichromate.

3.2. Alterations in hippocampal oxidative stress biomarkers:

Here we examined the impact of PDC on oxidative stress indicators (MDA and catalase) produced in brain hippocampi with and without AD. As clear from figure (3A) that LPS resulted in promoting MDA formation by 3.59 folds when compared to normal control. It is also obvious that PDC injection lead to significant increase in MDA formation in hippocampi of AD rats, by 4.63 folds when compared to normal control and 1.29 folds when compared to AD control rats. When compared to PDC alone, the third group showed 1.17-fold significant increase in MDA levels. As presented in figure (3B), injection of LPS lead to increase in catalase activity in rats' hippocampi by 1.23-folds when compared to normal control. It is also obvious that PDC injection resulted in significant elevation in catalase activity in rats' hippocampi by 2.11-folds when compared to normal control and 1.71-folds when compared to AD control rats. Moreover, when

PDC was injected to AD rats it resulted in 1.2-folds significant increase than PDC alone.

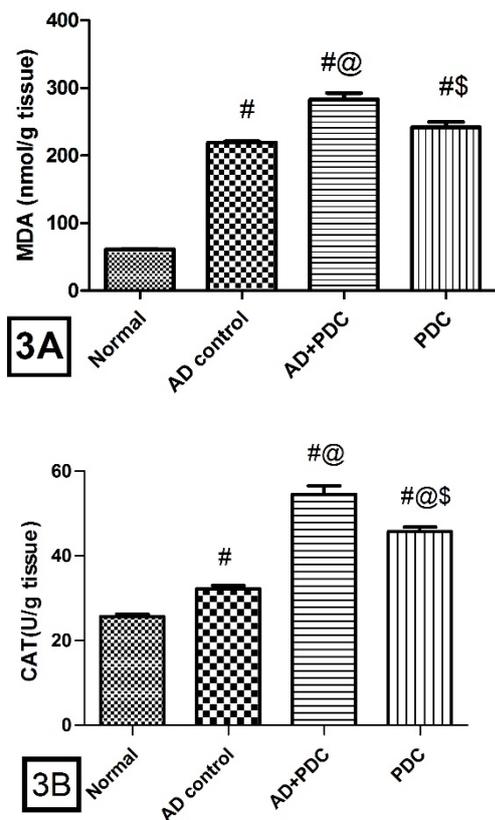


Figure 3. PDC induced oxidative stress in brain hippocampal tissues. (3A) MDA content in brain hippocampal tissues from rats treated with either saline (normal control), LPS (AD control) or LPS+PDC was determined using a TBARS assay kit. Data represent means \pm S.E.M. (n=6). [#] Significantly different from normal control group. [@] Significantly different from AD control group. ^{\$} Significantly different from AD+PDC group. (3B) CAT activity in brain hippocampal tissues from rats treated with either saline (normal control), LPS (AD control) or LPS+PDC was determined using a H₂O₂ assay kit. Data represent means \pm S.E.M. (n=6). As compared with normal control ([#]), AD control ([@]) and AD injected with PDC (^{\$}). All groups were compared at p < 0.001. MDA: malondialdehyde, CAT: catalase, AD: Alzheimer’s disease, PDC: potassium dichromate.

3.3. Alterations in bone marrow micronuclei and polychromatic erythrocytes count:

The results of Micronucleus assay (MN) were done on 4 rats per each group, where 2 slides were assayed for each rat (figure 4). As depicted from figure (5A), when talking about polychromatic erythrocytes, it is shown that LPS alone didn’t result in significant difference from normal control, but injection of PDC resulted in significant elevation of PE% by 1.79 folds when compared to normal, 1.64 folds when compared to AD rats, and 1.11 folds when compared to PDC alone. On the other hand, when talking about micronuclei (MN-PE%), a significantly surge was observed when comparing all groups to normal control group, by 3.81 folds in AD

control, 4.63 folds in AD+PDC, and 4.31 folds in PDC alone. However, no statistically significant variance among other groups when matched together. All these data are represented in table 1 and figure 5.

Table 1. PE, N and MN-PE count / 1000 cells/ slide in rat bone marrow.

Group	PE count / 1000 cells	N count / 1000 cells	MN-PE count / total PE
Normal control	199 \pm 10	801 \pm 10	2.4 \pm 0.32
AD control	218.94 \pm 9.4	782 \pm 9.4	7.6 \pm 0.53 [#]
AD+PDC	357 \pm 5.2 ^{#@}	643 \pm 5.2 ^{#@}	15 \pm 1.2 ^{#@}
PDC	321.8 \pm 7.8 ^{4#@}	678.3 \pm 7.8 ^{4#@}	12.88 \pm 0.7 ^{4#@}

Data represent means \pm S.E.M. (n=8). [#] Significantly different from normal control group at p < 0.05 [@] Significantly different from AD control group at p < 0.05. ^{\$} Significantly different from PDC alone. PE: Polychromatic erythrocytes; MN-PE: Micronucleated polychromatic erythrocytes; AD: Alzheimer’s disease; PDC: potassium dichromate.

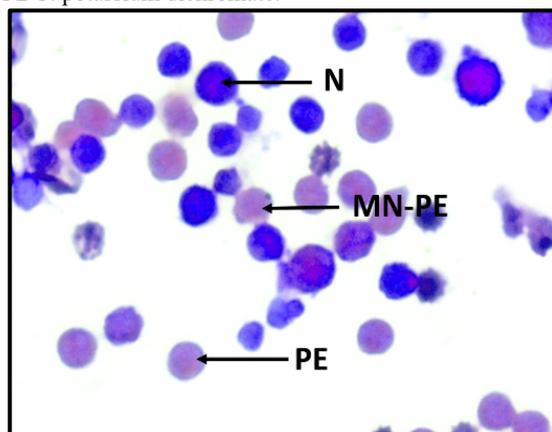


Figure 4. Bone marrow cells: nucleated cells (N), polychromatic erythrocytes (PE), and micronucleated-polychromatic erythrocytes (MN-PE).

3.4. Alterations in features of CA3 hippocampal tissue and its number of surviving neurons:

An additional expression of the aforementioned biochemical and cytogenetic outcomes, figure 6 illustrates the changes in the photomicrographs of the H&E stained sections of the various injections (from B to D) when related to normal tissue sections (A) of the intact CA3 region of brain hippocampi. These results were summarized in figure 7 (A to D), where staining with the Nissl stain showed the differences in surviving neurons throughout the different groups. The AD+PDC revealed minimal count of surviving neurons. These results are also presented graphically in figure (8).

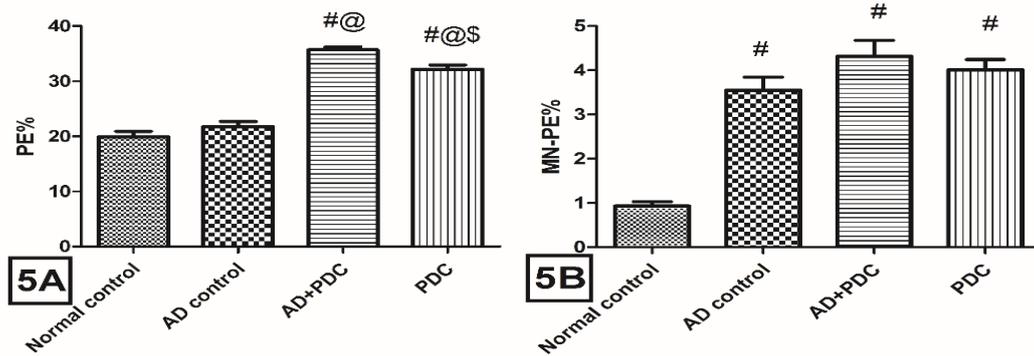


Figure 5. PE% & MN-PE% in rat bone marrow. (5A): PE%, (5B) MN-PE% in bone marrow from rats treated with either saline (normal control), LPS (AD control) or LPS+PDC was determined using Micronucleus assay method microscopically. Data represent means \pm S.E.M. (n=8 slides/ group). As compared with normal control (#), AD control (@) and AD injected with PDC (\$). All groups were compared at $p < 0.05$. PE: polychromatic erythrocytes, MN-PE: micronucleated polychromatic erythrocytes, AD: Alzheimer's disease, PDC: potassium dichromate.

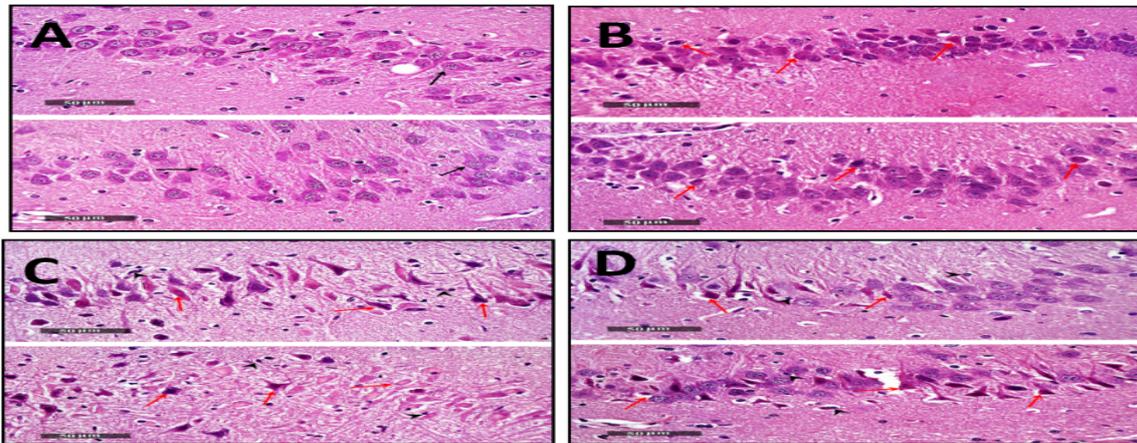


Figure 6. Alterations in histopathological photomicrographs of CA3 hippocampal region. *Normal Control (A) samples* demonstrated normal histological structures of hippocampal layers with apparent intact polymorphic and pyramidal neurons with intact nuclear and subcellular details (black arrow). Intact intercellular matrix was observed with normal glial cells infiltrates. *Alzheimer's disease control (B) samples* showed moderate records of necrotic and degenerated pyramidal neurons (red arrow) alternated with apparent intact neurons with moderate perineuronal and intercellular edema of brain matrix. *Alzheimer's disease intoxicated with potassium dichromate by single i.p. injection (C) samples* revealed abundant severe records of degenerative and necrotic pyramidal neurons (red arrow) alternated with few apparent intact cells, and accompanied with severe intercellular edema and vacuolization of brain matrix with mild records of reactive glial cells infiltrates (arrow head). *Potassium dichromate single injection (D) samples* showed milder records of degenerated and necrotic neurons in comparison with Alzheimer's disease intoxicated with potassium dichromate samples, with higher records of apparent intact neurons. (H&E; 50 μ m).

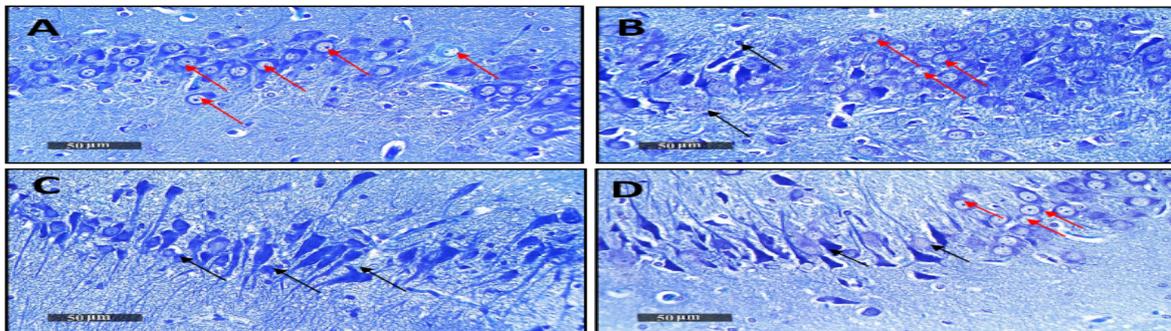


Figure 7. Effect of different groups on neuronal survival count. The Nissl stain reveal a decrease in the number of viable neurons in (B) Alzheimer's disease control, (C) Alzheimer's disease injected with potassium dichromate, and (D) injected with potassium dichromate only, compared to the (A) normal control group. (Nissl stain; 50 μ m). Red arrow: intact neurons, black arrow: damaged neurons.

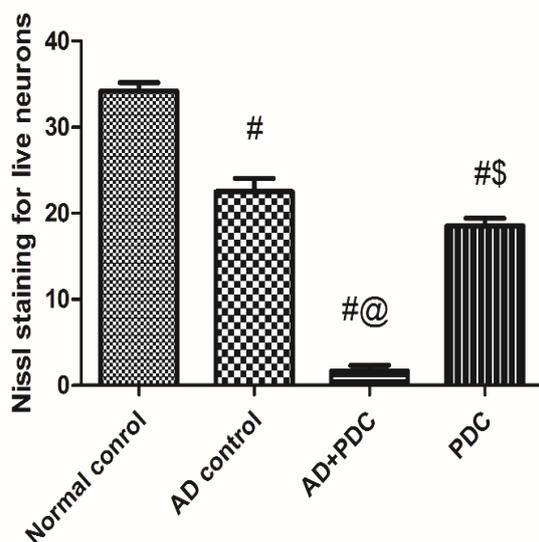


Figure 9. Show the living neurons count. Data are introduced as mean \pm S.E.M (n=4). Statistical analysis was done by one-way ANOVA then Tukey's multiple comparison test. When compared with normal control (#), AD control (@), and AD+PDC (\$) groups at $P < 0.001$. AD: Alzheimer's disease, PDC: potassium dichromate.

4. DISCUSSION

This research aimed to highlight the possible potential brain injury and cytogenotoxicity among individuals suffering from Alzheimer's disease (AD), after the exposure to Cr^{6+} dust environmentally or occupationally, in the form of potassium dichromate (PDC) compound. The hippocampus is a brain region, that consists of 3 fields, namely CA1, CA2, and CA3 in addition to the subiculum. A transfer of information occurs from dentate gyrus to CA3 then incorporated in CA1³⁰. Lately, the CA3 brain hippocampal region has attracted foremost consideration for its explicit role in memory practices, vulnerability to seizures and neurodegenerative processes.³¹

The previous reports stated the association between Alzheimer's Disease and the significant diminish in brain hippocampal and cortical acetylcholine (ACh) content, as a result of elevated acetylcholinesterase (AChE) synthesis and activity¹⁰. That's why we used AChE gene expression in brain hippocampus as a marker of AD induction. The present study annotated the histopathological alterations that may occur within CA3 region in the brain hippocampus, as a result of injection of PDC.

The brain, with its great oxygen-consuming and lipid-generous levels, greatly permits oxidative stress. Therefore, oxidative stress-induced damage to the brain has a robust possibility to negatively influence the CNS purposes³². Lipid peroxidation is the most noticeable feature in which degenerative change is most pronounced in the AD brain³³.

Catalase breakdown the toxic metabolic by-product hydrogen peroxide, into water and oxygen³⁴. As aforementioned in earlier studies, catalase is a major detoxifying enzyme found in the peroxisomes of the brain³³. Unfortunately, the brain possesses 50 times less catalase when compared to hepatocytes³⁵, so it is far more prone to oxidative stress.

For the last 4 decades, the *in vivo* micronucleus assay (MN) has turned out to be one of the most reliable methods to evaluate the cytotoxicity and genotoxicity of numerous physical and chemical elements²⁰. It was confirmed by many studies that an elevation in the micronuclei frequency is associated with several neurodegenerative diseases³⁶. The MN test has some advantages over other DNA damage detection techniques: (i) faster; (ii) cheaper; (iii) simpler than chromosomal aberration assessment. Although the MN assay cannot give information about chromosomal damage type, it is informative when the exposure causes aneugenic effects. By engaging all these features, this methodology becomes highly applicable in routine mutagenesis studies³⁷.

Lipopolysaccharide (LPS) is a crucial element in Gram-negative bacteria cell wall and has the ability to result in sepsis, shocks and microcirculation disturbances³⁸. Our results showed significant rise in AChE gene expression in groups injected with LPS. This is compatible with the results of Ming *et al.*³⁹ and Tyagi *et al.*⁴⁰. In addition, the results showed upsurged malondialdehyde (MDA) indicating elevated oxidative stress in the brain hippocampus of LPS-injected rats. This is compatible with the results of Mokhtari-Zaer *et al.*⁴¹ and Bargi *et al.*⁴². Furthermore, the catalase activity is boosted in LPS-rats a result of an adaptive response to the oxidative stress³⁴. İlce *et al.*⁴³ showed that LPS-single injection increased the MN instance, and that was the same as our results with LPS-injection for one week. In our study, LPS didn't exert any cytotoxic significance from normal control, as demonstrated by PE%. This concludes that LPS has genotoxic but not cytotoxic effect. But this is in contrast with the findings of Sharifi *et al.*⁴⁴, who stated that LPS enhanced expression of apoptotic proteins compared to controls.

Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) is a toxic and hydrophilic heavy metal, that is mobile in the natural environment⁴⁵. It is one of the most severe chromium compounds. It increases neurobehavioral disturbances in humans and experimental animals⁴⁶. In our present study, the rats were intraperitoneally intoxicated with PDC for once after induction of AD. This injection elevated the AChE gene expression in the hippocampus of AD rats, proving its ability to pass blood brain barrier, as mentioned by Salama *et al.*⁵. Our results showed that a single PDC administration induced brain oxidative stress in rats as evidenced by the increase in MDA content when

compared to normal rats and AD rats, as shown by Salama *et. al*⁵. Moreover, the observed increment in catalase activity level in groups injected with PDC may be justified that it is a defense mechanism against chromium-induced oxidative stress by enhancing the antioxidant enzyme activity. Since MN arises from DNA-breaks, Cr (VI) itself is non-reactive with DNA, though, during its reduction, the produced metabolites radicals can afterwards attack macromolecules, ending with multiform DNA damages, as strand breakage³⁴. Single injection of PDC significantly elevated the PE% when compared to normal and AD rats. This indicates its cytotoxic effect. In addition, PDC injection elevated MN% higher than normal, but no significant increase more than AD rats. These findings showed that PDC exerted the same genotoxicity as LPS.

5. CONCLUSIONS

This study showed that PDC exposure would be an extreme harm to AD rats, decreasing their ACh, increasing their brain oxidative stress, and cytogenotoxic levels by increasing the MN% and PE%. These results confirm its ability to deteriorate the AD case.

Funding statement: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethical Statement: The protocol followed in this study was approved by Al-Azhar Faculty of Pharmacy (Girls) scientific research ethical committee (no. 97, 2016).

Author contribution: This work was carried out in collaboration between all authors. Iman Hassan Ibrahim and Ezzat Ibrahim Aboul-Ela designed the study. Marwa Ashraf Essa performed the experiments and wrote the manuscript. Iman Hassan Ibrahim, Ezzat Ibrahim Aboul-Ela and Marwa Ashraf Essa analyzed the data and revised the manuscript.

List of abbreviations: PDC: Potassium dichromate; AD: Alzheimer's disease; LPS: Lipopolysaccharide; AChE: Acetylcholinesterase; ACh: Acetylcholine; MDA: Malondialdehyde; CAT: Catalase; MN: Micronucleus; MN-PE: Micronucleated polychromatic erythrocytes; PE: Polychromatic erythrocytes; N: Nucleated cells; A β : Amyloid β .

Authors consent for publication: We, the authors, give our consent for the publication of this research

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