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# Genotoxic Effect Of Acute Hexavalent Chromium Exposure On Alzheimer's Disease Induced In Rats

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***Abstract***
***Background:*** *Alzheimer's disease (AD) is a neurodegenerative disease, among its environmental risk factors is pollution with heavy metals.*
***Aim of the study:*** *investigate the genotoxicity of potassium dichromate (PDC) on rats with AD.*
***Material and methods:*** *Forty rats were equally divided into 4 groups: control group received normal saline intraperitoneally (i.p). for 7 days, AD group received lipopolysaccharide (LPS) 250µg/kg i.p. for 7 days, AD group intoxicated with potassium dichromate (PDC) received LPS 250µg/kg i.p. for 7 days, followed by single i.p. PDC injection (10mg/kg) one hour after the last LPS injection, PDC group received normal saline i.p. for 7 days followed by single i.p. PDC injection (10mg/kg) one hour after the last saline injection. Behavioural activity was assessed by open field test, oxidative stress was assessed by malondialdehyde (MDA) and catalase (CAT) levels in the brain hippocampus, while chromosomal aberrations were investigated in bone marrow for detection of genotoxicity.*
***Results:*** *AD and AD+PDC groups showed a significant reduction in locomotion compared to control group, while PDC group showed the sharpest reduction compared to the other three groups. MDA and CAT levels were significantly elevated in all groups compared to the control group, where the highest elevation was in the AD+PDC group. The chromosomal aberration% was high in all groups compared to the control group, in which AD+PDC group showed the highest percentage.*
***Conclusion:*** *Acute PDC exposure aggravates lipid peroxidation and genotoxicity in the AD rat model.*
*Keywords:* ***Alzheimer's disease; hexavalent chromium; chromosomal aberration; genotoxicity, oxidative stress.***

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## I. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is clinically characterized by a decrease in cognitive function and memory loss **(1)**. AD was first described by Alois Alzheimer in 1906. It accounts for 60-80% of dementia cases worldwide **(2),** where 47.5 million people are living with dementia, and nearly 7.7 million new diagnoses are made every year. The observed rise of AD is expected to inflict a rising financial and social burden worldwide **(3)**. The prevalence of all causes of dementia worldwide is predicted to be increased from 50 million in 2010 to 113 million people by 2050 **(4)**.

The most typical AD symptom is short-term memory problems, although other symptoms include impairment in visuospatial processing, expressive speaking, and executive (mental agility) functions also occur. The majority of AD cases are not dominantly inherited, and many AD cases have a complex relationship to genetics **(5).**

Many hypotheses were proposed as possible causes for AD. Among them, the cholinergic dysfunction and alteration in the generation and processing of amyloid β (Aβ) protein hypothesis are believed to be the major cause of AD. However, there isn't a widely accepted theory describing the pathophysiology of AD at this time **(6)**.

Several risk factors for AD involve aging, genetic factors, nutrition, and environmental pollutant including heavy metals. Aging is the most crucial risk factor for AD. Young persons, seldom develop AD, and the majority of cases begin later in life, usually around the age of 65 **(7)**. Aging is regarded as an irreversible complex process that affects many organs and cell systems accompanied by a decrease in the weight and volume of the brain, synapses loss and enlargement of ventricles in specific areas with deposition of senile plaques and neurofibrillary tangles. Moreover, several conditions such as dysregulation in cholesterol homeostasis, glucose

hypometabolism, dysfunction of mitochondria, depression, and decrease in cognition might appear during aging **(8).**

Approximately 70% of AD cases were associated with genetic factors. The majority of early-onset AD cases are inherited in an autosomal dominant pattern, and mutations in dominant genes such as presenilin 1, presenilin 2, apolipoprotein E, and amyloid precursor. Associated with proteins associated with AD **(9, 10)**. However, genetic risk factors and aging cannot elucidate all cases of AD. Air pollution, infectious diseases, diet, metals, and many other environmental risk factors induce inflammation and oxidative stress, increasing the risk of developing Alzheimer's disease. It has been shown that exposure to high levels of air pollution can damage the olfactory mucosa and bulb and frontal cortical regions. There are links between oxidative stress, neurodegeneration, and neuroinflammation and the presence of her Aβ plaques and hyperphosphorylated tau in the frontal cortex of air pollutant-exposed individuals. Air pollution can lead to increased Aβ formation and accumulation and impaired cognitive function **(11, 12)**. Several studies have been conducted on the relationship between diet and AD. Many dietary supplements such as vitamins, antioxidants, polyphenols, and fish have been found to reduce the risk of AD, but excessive caloric intake and saturated fat are associated with an increased risk of AD. **(13)**. Another risk factor for AD is malnutrition, in which deficiencies in some nutrients, such as vitamin D, vitamin B12, and folic acid may lead to a decline in cognitive function. Additionally, Alzheimer's patients suffer from eating and swallowing problems and this may increase the risk of malnutrition **(14).**

Oxidative stress (OS) is reported to be increased in the brain of people with aging. OS is caused by corruption in the balance of the redox state, including either excess generation of reactive oxygen species (ROS) or the dysfunction of the antioxidant system **(15).** OS can be involved in AD pathophysiology by several mechanisms, including neuronal mitochondria dysfunction, oxidation of macromolecules, production of ROS by the binding of metal ions to Aβ plaques, and upregulation of Aβ synthesis. These mechanisms lead to the death of dendritic spines, loss of synapse, and inhibition of long term potentiation, which lead to cognitive dysfunction as a result **(16).**

Chromosomal aberrations include changes in the number of chromosome (gains and losses) and in its structure (inversions, deletions, and exchanges). Many of these aberration types can be observed when chromosomes be viewed by standard light microscopy **(17).** The presence of variations in chromosome number (i.e. Aneuploidy or loss/gain of whole chromosomes) in AD individuals has been debated for many years **(18, 19)**. However, the link between numerical abnormalities of the chromosome and AD pathogenesis has remained a matter of conjecture. There are some early studies that reported chromosomal aberrations in AD **(20-23)**.

Metals that are present in nature and biological systems are classified into bio-metals (e.g., iron, copper, and zinc) that have a physiological roles in living cells, and toxicological metals (e.g., aluminium and lead) that do not have any biological function **(24)**. Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. Human exposure to heavy metals is a global public health problem **(25).** Arsenic, lead, chromium, cadmium, and mercury are among the priority metals that are significant in public health due to their toxicity. Even at lower exposure levels, these metals are regarded as systemic toxins that can cause damage to multiple organs **(26)**.

Metals and metalloids play crucial role in the development of AD. Because of Aβ is a metalloprotein, zinc, copper, and iron enhance Aβ aggregation and plaque formation **(27-29)**.

Chromium (Cr) is a naturally occurring element that is found in the Earth's crust with valency s ranging from Cr (II) to Cr (IV), but due to both natural and anthropogenic activities, Cr can enter air, soil and water **(30).** At low concentrations, Cr is used for medical purposes as dental implants, appliances and tools. However, at high concentrations, it is toxic and carcinogenic **(31).** Cr compounds can enter the body via ingestion in food and water, or pass through the intranasal cavity, which is enriched with highly vascularized and a highly permeable membrane **(32).**

Hexavalent chromium Cr (VI) is highly mutagenic and linked to different types of cancers including prostate, renal, bone, lymphoma, leukemia, brain, gastrointestinal, and lung cancers **(33)**. The carcinogenic and mutagenic nature of Cr may be explained by the various genotoxic effects that it produces; Cr forms DNA adducts, causes DNA strand breaks, DNA intra- and inter-strand crosslinks, and DNA-protein crosslinking **(34)**. Very recent evidence revealed that selenium reduces the risk of Alzheimer's disease, whereas Cr increases the risk **(35)**.The effect of Cr on chromosomal aberration was early studied**.** Chromosome-breaking activity was significantly higher for the compounds with hexavalent than trivalent Cr **(36)**.

**The aim of the study:** The main aim of this work is to study the genotoxicity of Cr (VI) on rats with Alzheimer's disease.

## II. Materials and methods

***Animals:***

Forty male Wistar rats aged 8-12 weeks with initial body weight of 180-220 g, were used in the present study. The animals were supplied from the animal house colony of The Egyptian Organization for Biological

Products and Vaccines (Cairo, Egypt). Rats were housed in good ventilated standard polypropylene cages (3-4 rats / cage) in the animal house of Faculty of Pharmacy, October 6 University, Egypt, and kept under controlled conditions of 25 ± 2 ◦C room temperature, 50–70% humidity and 12 hours of light / dark cycle. The rats were provided with standard diet pellets (20% protein, 53% corn starch, 10% sucrose, 5% fiber, 7% soybean oil, and 5% vitamin mixture) purchased from the modern veterinary office (Giza, Egypt) and water *ad libitum*.

All experimental procedures and animal handling were performed according to the Guide for the Care and Use of Laboratory Animals and were approved by the Scientific Research Ethical Committee of the Faculty of Pharmacy (Girls), Al-Azhar University, Egypt, for animal use (no. 97/ 2016).

***Chemicals***

Potassium dichromate (PDC) as a source of Cr (VI) was supplied from Alpha Chemika, India, while lipopolysaccharide (LPS) was purchased from Sigma-Aldrich, USA

***Experimental design***

Rats were divided into 4 groups (10 rats / group) as follows:

- Group 1 (Normal control group): received normal saline i.p. for 7 days.
- Group 2 (AD group as positive control): received LPS 250 µg/kg. i.p. for 7 days **(37).**
- Group 3 (AD + PDC group): received LPS 250 µg/kg i.p. for 7 days, followed by 10 mg/kg PDC as a single i.p **(38)**. PDC injection one hour after the last LPS injection.
- Group 4 (PDC group): received normal saline i.p. for 7 days and then were i.p. injected once with PDC (10 mg/kg b.wt.) one hour after the last saline injection.

Four hours after the last received dose, rats were subjected to an open field test for assessment of their behavior and locomotor activity. Twenty hours later, rats were anesthetized, then sacrificed, and subjected to whole brain and femur separation. This design is summarized in **figure (1)**.

The brain was perfused with phosphate-buffered saline solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots, then blotted dry. The two hippocampal parts from each brain were dissected out, and quickly stored at -80 ºC for later determination of biochemical parameters.

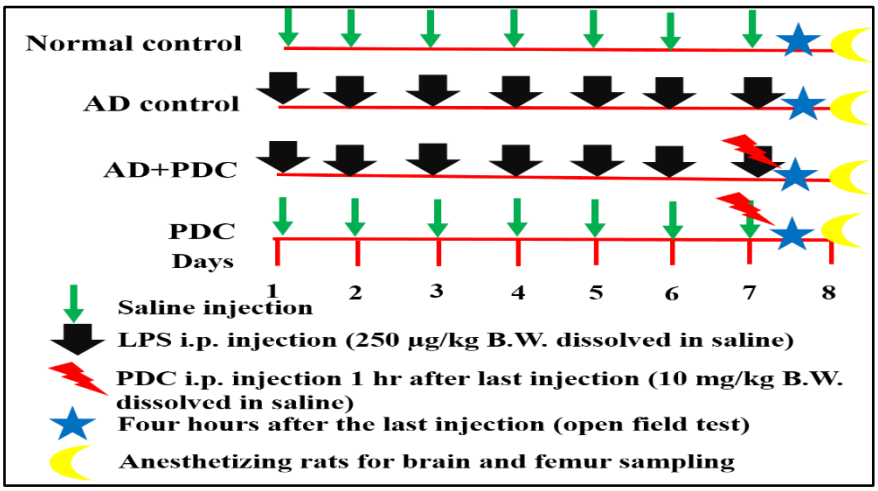


**Figure (1): Summary of the study design**

***Behavior assessments (open field test)***

Four hours later from the last received dose, each rat was placed in the open field apparatus that consisted of a square area (76 × 76 cm) with 42 cm high walls. The floor of the arena was equally divided by the help of lines into 25 equal squares. To assess the locomotor activity, the rat was placed in the central square of the arena and allowed to explore the area. The number of squares crossed by animal with its four paws was counted for 5 min as reported earlier **(39)**. After each test session, the apparatus was carefully cleaned and deodorized with 70% alcohol solution for accepting a new rat.

***Biochemical investigation***

To evaluate the oxidative stress level in hippocampus, one hippocampus part was homogenized in 5 ml cold potassium phosphate buffer, pH 7.4, forming 20% homogenate, which was then centrifuged at 4,000 rpm for 15 minutes. Catalase (CAT) activity was determined in brain hippocampal homogenate, using CAT colorimetric

assay **(**Biodiagnostic, Egypt**)** according to the method of Aebi, 1984 **(40).** The activity of CAT was expressed as U/g tissue. While lipid peroxidation was assessed in brain hippocampus by measuring the levels of Malondialdehyde **(**MDA) "the end product of lipid peroxidation" using thiobarbituric acid (TBA) test according to Ohkawa *et al*. **(41)** , in brief, 10% tissue homogenates were added to 3 ml of 1% phosphoric acid, and 1 ml of 6% TBA and then the mixture was heated in boiling water bath for 45 min. After cooling, 4 ml of n-butanol was added to the mixture, then vortexed for 1 min, and centrifuged for 10 min at 3000 RPM. The supernatant was transferred to a test tube and the absorbance was read at 532 nm using a spectrometer. The calibration curve of tetraethoxypropane standard solutions was used to determine the concentrations of TBA–MDA adducts. The MDA level was expressed as nmol/g of tissue.

***Chromosomal aberrations assay:***

Rats were injected i.p. with 0.5% colchicine (0.5%, 0.5 ml/rat) 24 hours after the last injection. Three hours later, rats were killed and their bone marrow from both femurs were collected in a tube, suspended in hypotonic solution (0.56% KCl) and incubated for 20 min at 37$^{o}$C followed by centrifugation at 1500 rpm for 5 min.

The cell pellets were fixed twice by suspension in freshly prepared Carnoy's fixative solution (methanol: glacial acetic acid in ration of 3:1 *v/v*). A few drops of the fixed cell suspension were spread onto a microscope slide. Five slides were prepared for each rat, and then slides were allowed to dry on a wormed hot plate at 55$^{o}$C. The air-dried slides were stained by immersion in 10% Giemsa solution in phosphate buffer (0.5 M $Na_2HPO_4$, pH 6.8) for 10 min. Slides were examined under light microscope, scanned for mitotic metaphase spreads by magnification objective with a 25X, and analyzed with a 100X oil objective lenses. A hundred well spread metaphases were analyzed per rat. Metaphases with structural and numerical aberrations (chromatid gaps, chromatid breaks, acentric chromosome, ring chromosome, chromosomal fragments, centromeric fusion and polyploidy) were recorded. Mitotic index (the number of cells undergoing mitosis divided by the total number of cells) was calculated according to the following formula: mitotic index (MI) = number of dividing cells X 100/total number of cells (1000/group).

***Statistical analysis of data:***

All data analysis was performed using Prism 5 for windows version 5.01 (GraphPad software Inc., La Jolla, CA, USA). Multiple comparisons were achieved using analysis of variance (ANOVA) followed by Tukey test as post host test. The $p$ value $\leq 0.05$ was regared as statistically significant.

## III. Results:

***Effect of PDC on the behavioral changes:***

Results revealed significant differences in crossing squares between groups subjected to PDC injection. A profound decrease in locomotor activity was found when comparing AD+PDC with normal control reaching 0.43-fold. A significant decrease in crossing squares was found when PDC was compared to AD+PDC, reaching 0.57-fold, however there is a reduction in locomotor activity in AD+PDC when comparing AD positive control group, but the reduction is not statistically significant. **(Table I)**.

**Table (I): Effect of PDC on locomotor activity of the rats with LPS-induced AD in OFT:**

| **Group:** | **Normal** | **AD control** | **AD+PDC** | **PDC** |
|---|---|---|---|---|
| **No. of squares crossed/ 5 min** | **126±9.3** | **60±2.2** [a] | **54±1.9** [a] | **31±1.6** [abc] |

n= 10 rats/group

Values are expressed as mean ± S.E.M.

**a** Significantly different from normal control group at $p < 0.05$

b Significantly different from AD positive control group at $p < 0.05$

**c** Significantly different from AD+PDC groups at $p < 0.05$

***Effect of PDC on biochemical investigations in brain hippocampus:***

Activity of CAT was significantly increased in AD positive control group as compared with normal control by 1.28-fold ($p < 0.001$).

Significant increase in CAT activity in AD+PDC (1.68-fold) group was observed when compared to AD positive control at $p < 0.001$. Furthermore, the statistics revealed significant differences (at $p < 0.001$) between PDC alone when compared to its respective AD+ PDC groups, showing 0.83-fold increase **(Table II)**.

The concentration of MDA significantly increased in hippocampus tissue of rats with AD (3.59-fold) when compared with the control group. The increase in MDA concentration in AD+PDC was found to be

significantly increased by 1.28-fold when compared to AD positive control group. However, PDC exerted more lipid peroxidation when injected to AD rats than when injected alone, reaching 1.15-fold respectively ($p < 0.05$) **(Table II)**

**Table (II): Effect of PDC on CAT activity and lipid peroxidation in hippocampus tissue:**

| Groups<br>Parameters | Normal control | AD-control | AD+PDC | PDC |
|---|---|---|---|---|
| CAT (U/g tissue) | 25±0.43 | 32±0.61[a] | 54±1.5 [ab] | 45±0.93 [abc] |
| MDA (nmol/g tissue) | 61±0.87 | 219±1.5[a] | 281±7.2 [ab] | 243±5.9 [abc] |

n= 8 rats/group

Values are expressed as mean ± S.E.M.

**a** Significantly different from normal control group at $p < 0.001$

b Significantly different from AD positive control group at $p < 0.001$

c Significantly different from AD+PDC group at $p < 0.001$

***Chromosomal aberrations (CAs) assay findings:***

The LPS injection of the experimental rats had induced notable structural and numerical chromosomal aberration types in the bone marrow cells. LPS induced statistically significant chromosomal aberration record 19.6% with MI: 19.1, (6.12-folds) when compared to normal rats.

Injection of PDC to AD rats significantly increased the percentage of chromosomal aberrations reaching 36.2% with MI 14.9. All the percentages were statistically significant at $p < 0.001$ representing 11.3-folds of normal and 1.84-folds of AD control groups. The injection of PDC to AD rats induced notable both structural and numerical chromosomal aberration types in the bone marrow cells mainly chromatid gaps, breaks, acentric, and rings, as structural alterations, while fragments, centromeric fusion and polyploidy as numerical alterations.

Intraperitoneal injection of PDC to rats resulted in 26.6% chromosomal aberrations with MI 11.9. Chromosomal alterations represent significant ($p < 0.001$) difference from normal (8.31-folds), AD control (1.39-folds) and AD+PDC (only 0.73-fold) groups. The most noticed alterations were chromatid gaps, breaks, acentric, and rings as structural alterations, and fragments, centromeric fusion and polyploidy as numerical alterations **(Table III).**

**Table (III): Effect of hexavalent chromium on chromosomal aberrations and mitotic index in bone marrow cells of rats:**

| Parameters \ Groups | | Normal control | AD control | AD+PDC | PDC |
|---|---|---|---|---|---|
| Total number of examined metaphases/ (n) | | 500/5 | 500/5 | 500/5 | 500/5 |
| Count of chromosomal Aberration types | Chromatid Gaps | 2 | 16 | 25 | 18 |
| | Breaks | 1 | 13 | 26 | 18 |
| | Acentric | 2 | 12 | 22 | 18 |
| | Ring | Nil | 13 | 23 | 18 |
| | Fragments | 2 | 15 | 27 | 18 |
| | Centromeric fusion | Nil | 14 | 26 | 18 |
| | Polyploidy | Nil | 10 | 20 | 17 |
| Total abnormal metaphases | | 7 | 98 | 181 | 133 |
| Chr. Aberration% ±S.E.M.% | | 1.4±0.4 | 19.6±0.24[a] | 36.2±0.58 [ab] | 26.6±0.51 [abc] |
| MI | | 26.8 | 19.1 | 14.9 | 11.9 |

n= number of rats. Data are expressed as mean% ± S.E.M.%
**a** Significantly different from normal control group at $p < 0.001$
**b** Significantly different from AD positive control group at $p < 0.001$
**c** Significantly different from AD+ PDC groups at $p < 0.001$

## IV. Discussion:

Alzheimer's disease (AD) is the most common degenerative CNS disease in the elderly. It is predicted that 68% of increase in the global prevalence of dementia by 2050 will take place in low- and middle-income countries, where there is at present no evidence that the risk of AD and other dementias has been declining **(42)**. Notably, the main clinical manifestations are cognitive dysfunction, memory loss, and abnormal changes in personality and locomotion **(43)**. Genetics, along with other factors such as cerebrovascular diseases, diabetes, hypertension and obesity increase the risk of AD development **(44)**.

Lipopolysaccharide (LPS) is a vital element in Gram-negative bacteria cell wall and has the ability to result in sepsis, shock and microcirculation disturbances **(45)**. Previous analyses have shown that blood LPS levels in AD patients are 3-fold the levels in control **(46)**. LPS-induced neuroinflammation activates the mitochondrial apoptotic pathway and enhances neurodegeneration **(47).**

In the current study, Open field test was used to evaluate locomotor activity after LPS injection, where it resulted in significant decreased locomotor activity than in normal control rats, and this was in agreement with Bishnoi *et al.* **(48)**. From the behavioral point of view, AD patients often suffer from motor dysfunction, probably due to AD-related cortical and subcortical injuries. Moreover, it has been observed that patients with AD likely activate adaptive motor plans in order to control the complex interactions between cognitive and motor tasks **(49)**.

In the present study, levels of MDA and catalase were measured to assess the brain hippocampal oxidative state, where LPS-injected rats showed elevated MDA content, this harmonizes with the results of Bargi *et al*., **(50)** and Mokhtari-Zaer *et al*., **(51)**. Furthermore, the catalase activity was boosted in LPS-rats as a result of an adaptive response to the oxidative stress, and in contrast to Bargi *et al*., **(50)** and Aboulhoda *et al*., **(52)**. Although indirectly involved in AD pathophysiology, oxidative stress was further reinforced by the observation of elevated MDA and decreased glutathione, detected in brain cerebral cortices and hippocampi from AD rats **(52)**. Moreover, the brain, with its great oxygen-consuming and lipid-generous levels, greatly permits oxidative stress **(53)**. Lipid peroxidation, resulting in MDA production, is the most noticeable feature in the AD brain **(54)**. As aforementioned in earlier studies, catalase is a major detoxifying enzyme found in the peroxisomes of the brain **(54)**. Unfortunately, the brain possesses 50 times less catalase when compared to hepatocytes **(55)**, so it is far more prune to oxidative stress.

It was stated that patients with AD undergo chromosomal alterations at different levels, emphasizing the importance of these cytogenetic investigations in the routine management along with AD **(56)**. In the current study, rats with AD displayed significant percentage of chromosomal aberration with decreased MI when compared to normal rats. It was known that in AD there is a cell cycle defect, where chromosome instability and up to 30% aneuploidy occurs **(57)**. Cell cycle defect leads to mitotic inhibition, and revealed by decrease in MI which is defined as the percentage or ratio between the number of cells in a population undergoing mitosis to the total number of cells in a population **(58, 59)**. The MI is used to characterize the proliferating cells and to identify compounds that inhibit or induce mitotic progression depending on the proportion of the cell population that participates in the whole cycle of interphase **(60).** Collectively, chromosomal aberrations and decline in MI are the most sensitive indicators of bone marrow damage **(61).**

Potassium dichromate (PDC) is a toxic hydrophilic heavy metal, that is mobile in the natural environment **(62)**. Cr(III) is about 1000 times less toxic than Cr(VI) and it is the major form found naturally, while Cr(VI) in our environment is usually a result of human activity **(63).** As stated by Casalegno *et al*., the water-soluble Cr(VI) compounds as chromium trioxide, sodium chromate, sodium dichromate and PDC are acutely toxic to rats **(64)**. PDC is one of the most damaging Cr compounds, that increases neurobehavioral disturbances in humans and experimental animals **(65).** In humans, Cr (VI) excretion from plasma is generally rapid (within hours), while its elimination from tissues is slower (with a half-life of several days) **(66)**. Intraperitoneal injection of sub-lethal doses of chromate salts in rats and rabbits results in the presence of a substantial amount of Cr to the brain.

In the present study, the rats were intraperitoneally intoxicated with PDC for once to AD rats and normal rats to test its effect alone. This PDC single injection was equal to only 1/7.95 of the PDC LD50, that ranges from 46 to 113 mg/kg b.w/day for male or female rats **(64)**.

This single PDC-injection didn't significantly affect the locomotor activity of AD rats, however it was declined in normal rats receiving PDC alone, when compared to AD control rats. It can be concluded from this result that PDC alone is potent enough to reduce locomotor activity more than combined with AD. In line with the current findings, Hegazy *et al*., who found that behaviorally, the major toxic effects of intranasal PDC were observed on the locomotor and cognition functions. **(67)**.

Results of the present study showed that a single PDC administration induced brain oxidative stress and lipid peroxidation (LPO) in hippocampal tissues of rats as evidenced by the increase in MDA content when

compared to normal rats and AD rats. Similar results were obtained by Salama et. al., **(68)** and Patlolla *et al*., **(69)** in which The increasement in LPO may be due to the formation of hydroxyl radical through Fenton/Haber-Weiss reaction, catalyzed by chromium. This hydroxyl radical can abstract a hydrogen atom from a methylene group of polyunsaturated fatty acids to enhance LPO. Moreover, the observed increment in catalase activity level in groups injected with PDC may be understood as adaptive defense mechanism against chromium-induced oxidative stress by enhancing the antioxidant enzyme activity and this was in accordance with results observed by Patlolla *et al*. **(69)** and Hassan *et al*.**(70)**.

In agreement with Hassan *et al*. **(71)** , the present study revealed significant increase in several forms of chromosomal aberrations accompanied with a decline in MI, in the bone marrow cells of AD rats in acute PDC intoxicated groups, as compared to the normal and AD control groups. As observed by Nunes *et al*., there were chromosomal alterations in the peripheral blood lymphocytes in patients diagnosed with AD, highlighting the importance of the inclusion of cytogenetic investigations in the routine management of patients with AD **(72)**.

There is a link between LPO and chromosomal aberration in which LPO gives rise to two major by-products that are MDA and 4-hydroxy-2-nonenal (HNE). HNE was shown to cause micronuclei formation, chromosomal aberrations and sister chromatid exchanges **(73)**. Bird *et al*., reported that MAD is a genotoxic and induce chromosomal aberrations **(74)**. In a study carried out by Maeng *et al*., it was reported that genotoxicity including chromosomal aberrations and oxidative damage (plasma lipid peroxidation) were significantly higher in the Korean Cr-exposed workers than healthy persons **(75)**. So, we suggest that the high rate of chromosomal aberration observed in AD group intoxicated with PDC is due to the high level of LPO indicated by high level of MDA.

Finally, studying the effects of PDC on the chromosomal alterations in AD and, also their behavioral, biochemical impacts, led to uncovering the possible damaging effects that may result from exposure to this heavy metal, especially in AD patients.

## V. Conclusion:

Hexavalent chromium exposure aggravates oxidative stress and genotoxicity in Alzheimer disease.

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