CELLULAR CYTOGENETIC STUDY FOR TREATED ROLE OF SELENIUM AND OLIVE OIL AS AN-ANTIOXIDANTS AGAINST LEAD POISONING IN FEMALES MICE BONE MARROW CELLS

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ABSTRACT

The present study was designed to investigate the cellular cytogenetic effect of lead poisoning and the therapeutic role of selenium and olive oil as an antioxidant in mouse bone marrow cells.

Twenty one female mice, 12 weeks old, and of 20–25 gram weight were used. The female mice which divided into seven groups each group consist of 3 female mice ,the control group: oral giving by 0.9 % normal saline (N.S) daily for 28 days. The treated groups **A**,**B,C,D,E,F**: received orally with 50 mg/kg BW lead acetate, 50 mg/kg of lead acetates + o.5 mg/kg of selenium, 50 mg/kg of lead acetates +0.2 CC of olive oil, 100 mg/kg of lead acetates, 100 mg/kg of lead acetates+ o.5 mg/kg of selenium, 100 mg/kg of lead acetates+0.2 CC of olive oil daily for28 days respectively,

The administration of different doses of lead acetate caused a significant increase ($P < 0.05$) in number of chromosomal aberration in mouse bone marrow (ring chromosome, fragment, centric fusions and centromeric attenuation)the high dose caused more increase in number of chromosomal aberration in at 28 days especially ring form.

The treatment with antioxidants selenium (0.5mg/kg.B.W) and olive oil (0.2cc) caused decreased chromosomal aberration number.

Also, the results showed that lead acetate caused a significant decrease ($P < 0.05$) in the mitotic index parameter whereas the treatment with antioxidant caused an increase number of mitotic index in mouse bone marrow cells during 28 days.

INTRODUCTION

Lead is a wide-spread environmental pollutant, which has been implicated in toxic processes that affect several organ systems in man and other mammals(1). It is well accepted that lead intoxication is associated with decrease in the levels of endogenous antioxidants in liver, kidney, lung, heart and brain caused of anemia, hepato-renal dysfunction, genotoxicity and immune suppression (2; 3; 4;5).

Inorganic lead is ubiquitous in the environment because of natural origin and industryfactors. Lead is known to replace zinc in many enzymes, including those that are important for proper DNA metabolism and thus can cause fatal injury (6),however,the lead, cadmium, mercury and arsenic are among the main toxic heavy metals. That accumulated in food chains and has a cumulative effect (7). Heavy metals often have direct physiologically toxic effects and are sometimes permanently stored or incorporated in living tissues (8).

Lead is a metabolic poison and a neurotoxin that binds to essential enzymes and other cellular components and inactivates them (7). Toxic effects of lead are seen on hemopoietic, nervous, gastrointestinal and renal systems (9). Lead can induce single-strand DNA breaks, possibly by competing with metal binding sites in DNA (10).

The studies in animals showed that the significant increase in the chromosome aberrations in rate bone marrow cells after intraperitoneal administration of lead acetate in rats (11) and mice(12) . While,(13) did not find any increase in the frequencies of chromosomal aberrations in mice fed with lead acetate .

In human, most of studies using chromosomal aberrations test were performed in occupationally exposed workers, there is a considerable controversy regarding the ability of lead to cause chromosomal damage on exposed individual (14) . Some studies reported increases in the frequency of chromosomal aberrations in human populations exposed to lead (15;16).

However, other workers found no effects of lead exposure on chromosomal aberrations frequency (17;18). Although evidence of a genetic risk associated with lead exposure actually exists, there are still conflicting data on the conditions under which its genotoxicity becomes apparent. In addition, little is known about cytogenetic in rabbit

treated with lead acetate (19). In rats lead acetate cause chromosomal aberration (20).

This research aimed to detect the mutagenic effect of lead acetate that induced the chromosomal aberration in mouse bone marrow and protective role of antioxidant to reduce lead effect.

MATERIALS AND METHODS

Experimental design : Twenty one female mice, 12 weeks old and of 20-25 grams weight were used. The experimental animal divided to seven groups each group consist of 3 female mice as the following:-

Control group: oral giving with 0.9 % normal saline (N.S) daily for28 days.

treated groups: A, B, C, D, E and F: received orally with 50 mg/kg BW lead acetates, 50 mg/kg of lead acetates + o.5 mg/kg of selenium, 50 mg/kg of lead acetates + 0.2 CC of olive oil, 100 mg/kg of lead acetates, 100 mg/kg of lead acetates + o.5 mg/kg of selenium, 100 mg/kg of lead acetates+0.2 CC of olive oil daily for28 days respectively,

Cytogenetically analysis: Bone marrow from femur was obtained to perform analysis of chromosomal aberrations according to (21),

The animals were sacrificed 1–2 hr after injection of 0.5 mg/kg b.w. colchicines. Bone marrow preparation were made by extraction of bone marrow cell with 5–7 ml of KCl (0.65%) as a hypotonic solution at 37C^{o} for 25 min. The cells were centrifuged for 5 min. at 1000 rpm and then fixed in methyl acetic acid 3:1 and then re centrifuged at 1000 rpm for 5 min. The supernatant was discarded, the pellets resuspended in 5 ml of fixative and centrifugation repeated. The cells spread into clean slides were air dried stained with Giemsa stain solution and examined under light microscope at (1000X Oil Immersion).

Mitotic Index;

Determination the mitotic index according to method of (22) as in the formula:-

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total cells number}} \times 100$$

Statistical Analysis

Data analysis was done using a computer programme (SPSS 12.0 for windows). ANOVA (one way) and Duncan's multiple range test were used to compare between experimental groups. A value of $P<0.05$ was accepted as statistically significant.

RESULTS

Cytogenetic effect of lead in mice bone marrow cells

The present study was designed to investigate the possible protective role of Selenium and Olive Oil on bone marrow cells of mice exposed to different doses of Lead acetate. The chromosomal aberrations were measured by two end points as in the following:-

Effect of lead poisoning and treated role of selenium and olive oil in:-

Mice exposed to different concentrations of lead acetate were found to induce chromosomal aberration in the mouse bone marrow cells. The normal value of aberration in the control group was found to be significantly increased ($P <0.05$) in animals exposed to 50mg/kg B.W of lead acetate (Group A). For 28 days and in dose 100mg/kg B.W of lead acetate (Group D)the percentage again increased. Concerning the different types of chromosomal aberrations, it was clearly indicated that ring form are the most type of aberrations increased after lead administration. Fragmented chromosome, centric fusions, attenuation centromeric was also recorded

On the other hand, the mice which had treated with selenium as antioxidant contentious with lead acetate administration showed significant decrease ($P <0.05$) in the number of different type of chromosomal aberration in their bone marrow cells with different concentrations of Lead acetate in compared with control group **.**but in animal that had treated with olive oil contentious with lead acetate administration show significant increase ($P <0.05$) in the percentage of chromosomal aberration in compared with selenium addition that we can recorded in less level and in compared with control group**. Fig (1, 2, 3, 4 and 5)** (**Table 1**)

Mitotic index for effect of lead poisoning and treated role of selenium and olive oil:-

In this study the lead acetate dose 50 and 100 mg/kg B.W. Induced significant decrease ($P <0.05$) in the percentages of dividing cells in the bone marrow of female mice as compared with the control group, and the analytical essay shows the dose (100 mg/kg.B.W) was more effective on the decrease of mitotic index. whereas the mice which had treated with selenium and olive oil contentious with lead acetate administration showed

a significant increase (P <0.05) in the percentages of dividing cells in the bone marrow of female mice as compared with the group of lead acetate and control group (**Table 1**)

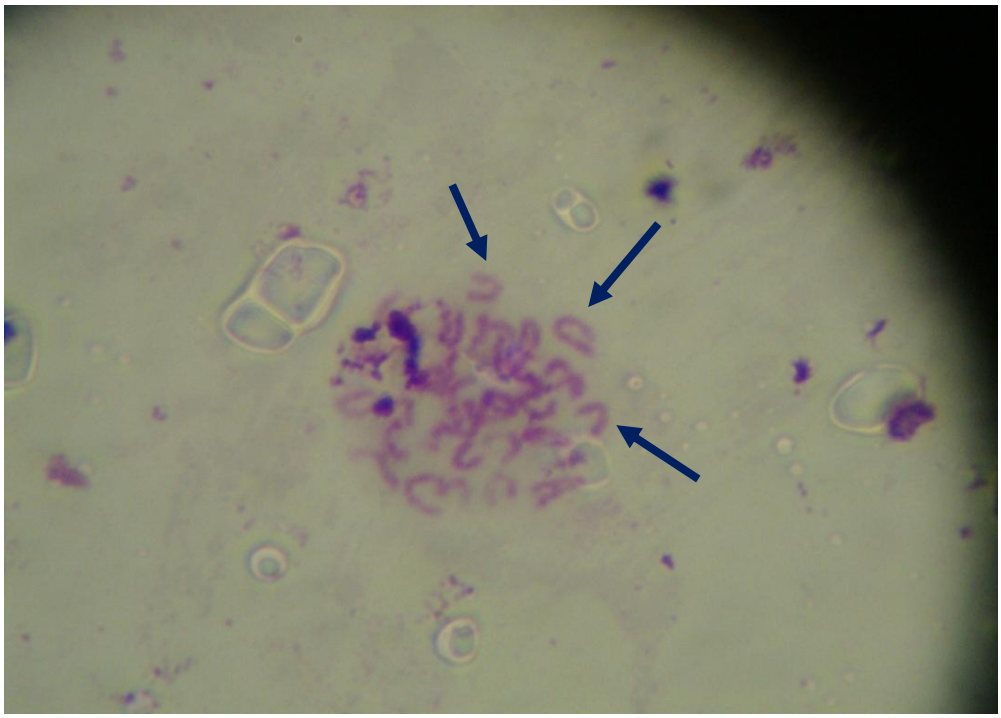


Fig: (1) Normal chromosome of metaphase in the control group(1000X Giemsa Stain)

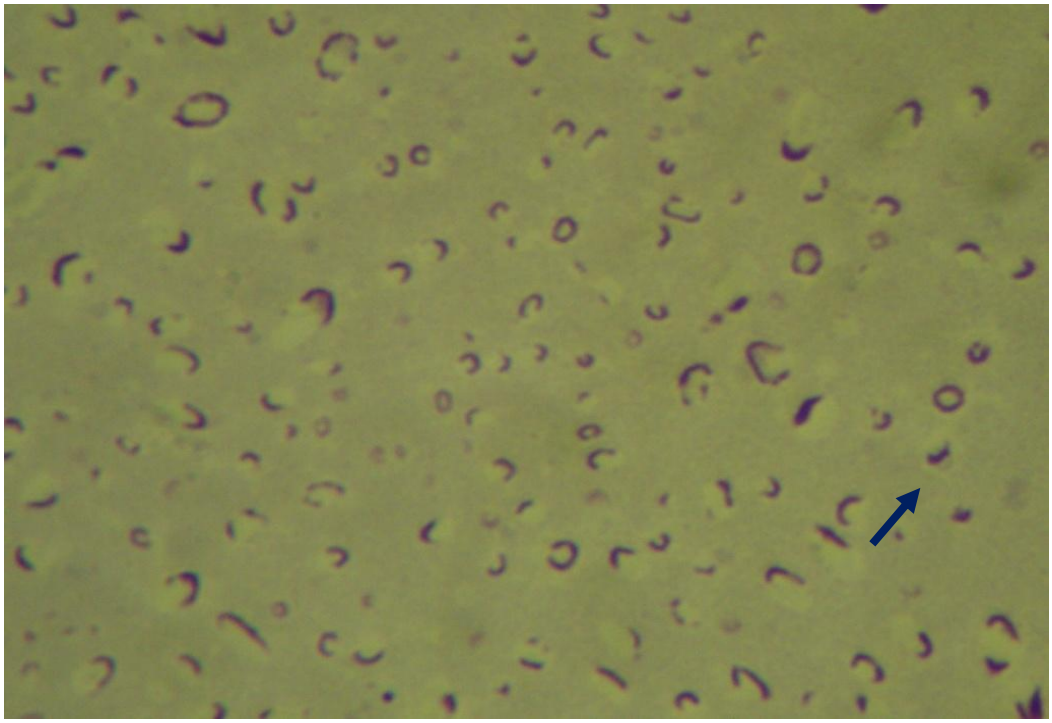
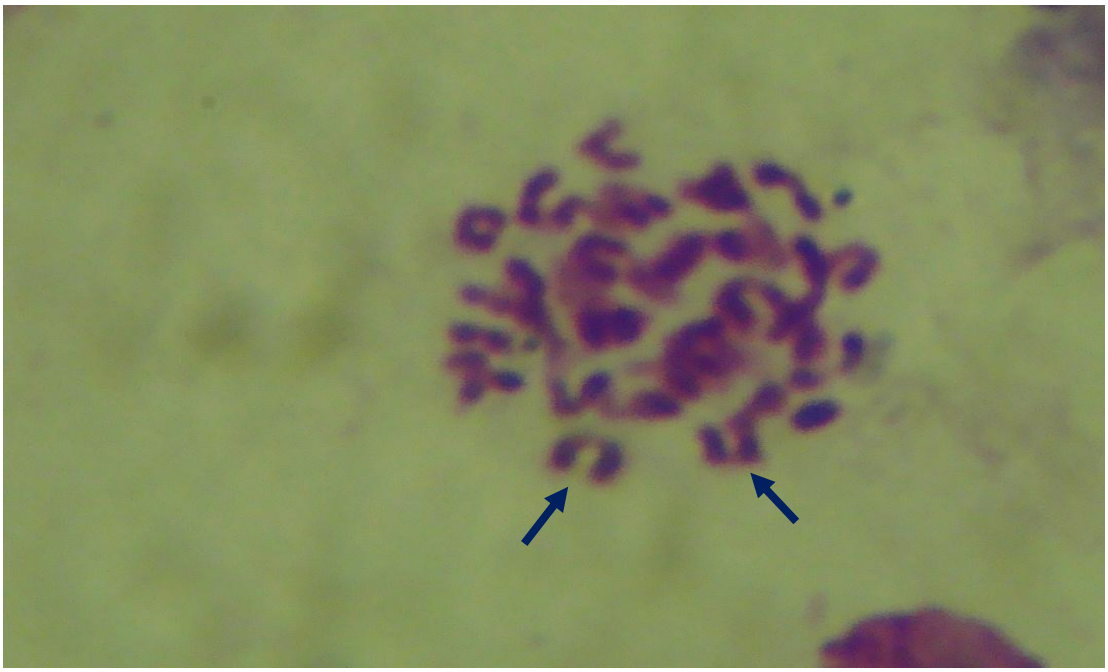
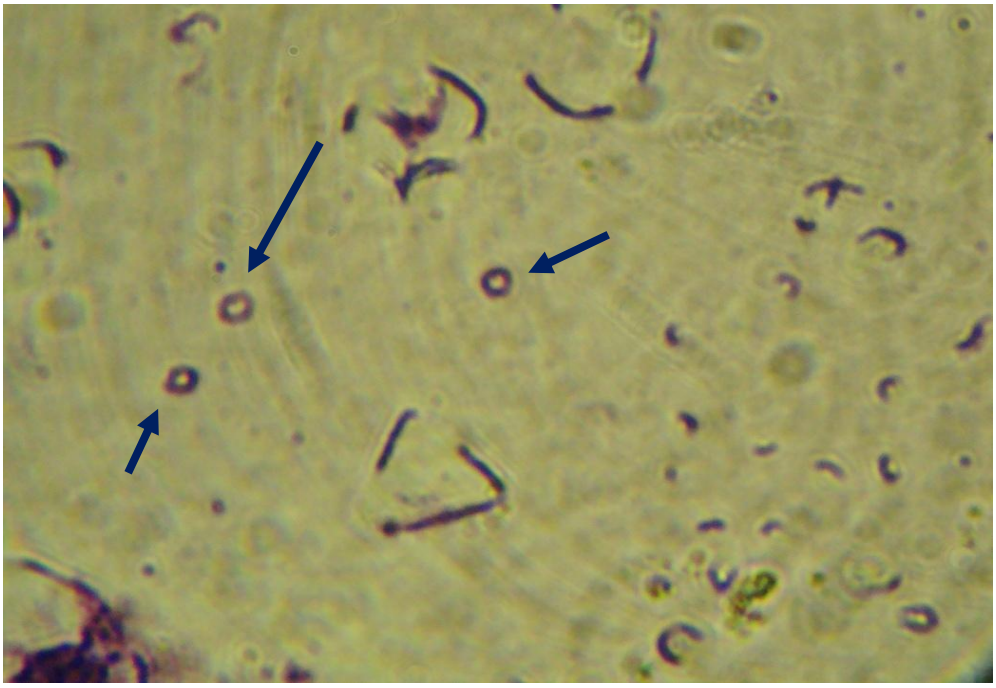


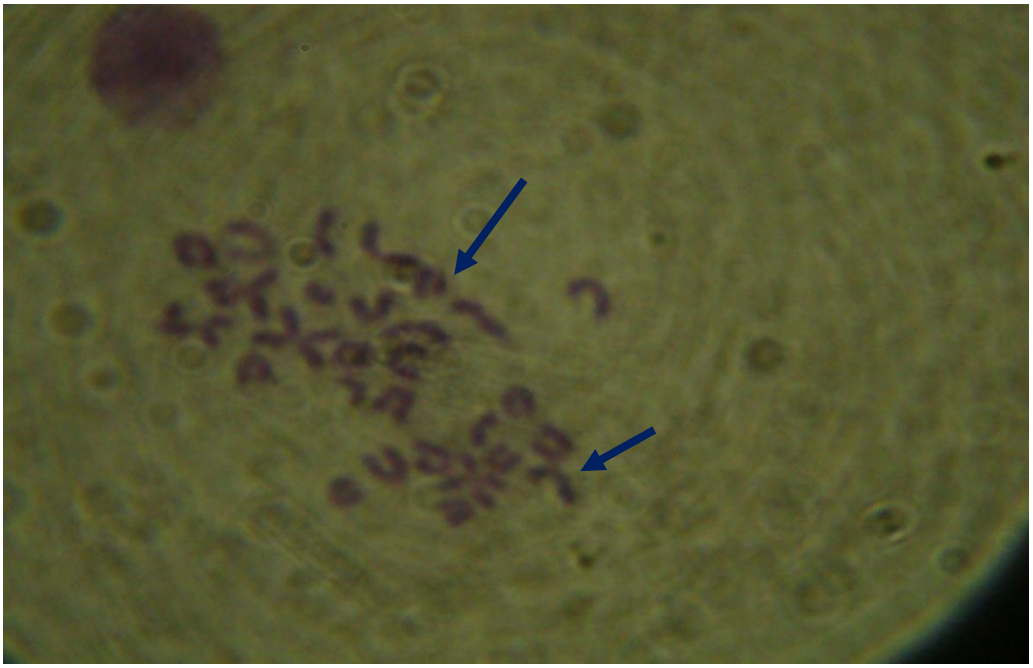
Fig (2) Chromosomal aberration type Fragmented chromosome exposure of 50mg/kg Lead acetate and olive oil (1000X Giemsa Stain)



Fig(3) Chromosomal aberration type Centric fusion exposure of 100mg/kg Lead acetate 1000X Giemsa Stain



Fig(4) Chromosomal aberration type Ring chromosome exposure of 100mg/kg Lead acetate & 0.2 mg/kg Selenium 1000X Giemsa Stain



Fig(5) Chromosomal aberration type Centromeric attenuation exposure of 100mg/kg Lead acetate 1000X Giemsa Stain

Table (1): The role of selenium and olive oil in chromosomal aberrations and Mitotic Index in bone marrow of female mice exposed to lead acetate.

Aberrations type / *Groups*	Ring	Fragmentation	Centric fusion	Centromeric attenuation	Mitotic index
Control (0.9 % N.S.)	a 1± 0.05 A	a 1.0±0.087 A	b 1.4±0.079 A	c 0.6±0.025 A	a 77.61±5.54
Group A	a 10.57±1.05 B	b 7.16±0.55 B	a 10.25±1.15 B	c 3.5±0.37 B	b 52.5±3.71
Group B	a 8.5± 1.71 C	b 5.88±0.8 C	b 9.0±1.11 Bc	c 2.71±o.41 B	ce 68.62±7.31
Group C	a 10.55±1.71 B	b 6.57±0.7 Bc	a 9.4±1.53 B	c 3.25±0.65 B	c 66.60±3.95
Group D	a 12.4±1.53 D	b 7.51±1.62 B	b 8.43±1.62 C	c 3.79±0.32 Bc	d 48.38±5.10
Group E	a 4.3±0.33 E	b 6.5±0.29 B	a 3.8±0.37 E	a 4.57±0.61 C	c 60.86±4.3
Group F	a 7.45±0.85 C	ab 6.8±0.67 B	b 5.81±0.77 D	c 2.4±0.51 B	e 58.7±7.81

*Significant differences at (P <0.05). Duncan's multiple range tests.

***Small letters refer to significant differences horizontally.**
***large letters refer to significant differences vertically.**
***S. E. Stander error**

DISCUSSION

The chromosome aberration frequencies in the analyzed bone marrow cells because bone marrow is one of the most convenient tissues for testing the mutagenic effects of environmental factors due to the low frequency of spontaneous chromosomal aberrations, high cell proliferative activity, relatively rapid and simple method of making the preparations. The cytogenetic method applied is intended to show the sensitivity of chromosomal aberration assay in animal's bone marrow to determine whether certain components of the environment (lead) can induce chromosomal aberrations with a frequency that is significantly higher compared with their frequency in the control animals.

The present study indicated that animals treated with lead acetate showed several-fold increase in the frequency of deferent type of chromosomal aberration and a significant decrease of mitotic index compared with control group. On the other hand the experimental animal treated with both selenium & olive oil showed a significant decrease in number of chromosomal aberrations and the mechanism of protection of Selenium &Olive oil may also depends on potentially scavenging the toxic and free radicals of lead acetate. In eukaryotic cells, this metal is usually studies reported increases in the frequency of genotoxic through a mechanism that until now has not been well characterized and possibly involves indirect damage to DNA, either by affecting the stabilization of chromatin or by interacting with repair processes (23;24)

Chromosomal rearrangements are sensitive endpoint towards the action of genotoxic with various origins. Chromosomal aberrations are changes in chromosome structure, which involve gross alteration of the genetic material and are detected using light microscopy. Structural chromosomal aberrations may be induced mainly by direct DNA breakage, by replication on a damaged DNA template, and by inhibition of DNA synthesis (25; 26). The application of cytogenetic observations is the various concentration of lead acetate causes the chromosomal aberration and significantly different from control. These observations clearly show that lead acetate is mutagenic, capable inducing chromosomal aberration.

Generally chromosomal aberration due to interfere of spindle fibers formation, which cause disturb of mitotic division. Chromosomal stickiness arise due to improper folding of chromosome fiber into single chromatid and chromosomes (27;28). Lead compounds do

not appear to damage DNA directly. However lead ions are known to participate in reaction generating ROS, which can cause DNA breaks, and damage of chromosome structure. ROS induced DNA alterations due to lead catalysis could not have been repair. In addition, leads ions are repeatedly known to inhibit the DNA polymerase _ one of the enzymes involved in DNA repair process (29;30). There are several possible mechanisms how lead might interfere with DNA repair process Besides a direct interaction with repair enzymes, lead ions may also interfere with calcium regulated processes involved in the regulation of DNA replication and repair (30).

In the present study, we have been intensively studied lead as carcinogens and we have investigated the suppressive effect of selenium and olive oil as an antioxidant - to reduce chromosomal aberrations caused by lead in mouse bone marrow cells. So we suggest that the extract of selenium & olive oil provide variable protection to genetic material indicated by reduction of the total aberrant cells or different types of chromosomal aberrations. This show that the present results are agreement with the observation of (31)which show that the oral administration of volatile oil after administration of lead acetate provides variable protection from its genotoxic effect and(32) which reported that the toxic metal may lower genetic stability by oxidative DNA damage and interaction with DNA repair processes and the present study agreement with (33)and(34)

دراسة وراثية خلوية للدور العلاجي للسلينيوم وزيت الزيتون كمضادات اكسدة ضد التسمم المستحدث بالرصاص في نخاع العظم للفئران المختبرية

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الخلاصة

الدراسة الحالية صُمّمتْ لتَحرّي التأثيرِالوراثي الخلوي للتَسَمّم بخلات الرصاص والدورِ العلاجّي للسلينيوم وزيت الزيتون كمضادات تأكسد في خلايا نُخاعِ عظم الفئرانِ.

21 من اناث الفاران بعمر 12 اسبوع ووزن 20-25 غم قسمت الى 7مجاميع تظمنت كل مجموعه 3 فاران، مجموعة السيطرة جرعت فمويا بالمحلول الفسيولوجي والمجاميع المعاملة (ا،ب،ت،ث،ج،ح)حيث تم تجريعها فمويا ب50 ملغم /كغم خلات الرصاص ،50ملغم من الرصاص+0,5 ملغم من السلينيوم ،50ملغم من الرصاص+0,2 مللتر زيت الزيتون ، 100 ملغم/كغم من الرصاص ، 100 ملغم من الرصاص+ 0,5 سلينيوم ، 100ملغم من الرصاص +0,2مللتر من زيت الزيتون بالتتابع يوميا ولمدة 28 يوم.

سبّب التعرض لجُرَعٍ مختلفةٍ من محلول خلات الرصاص لوحده زيادة في عددِ التشوهات الكروموسومية في نُخاعِ عظم الفئرانِ (كروموسوم حلقي، جزء، عمليات إنشطار مركزية وكرموسوم ذو سنترومیرين) الجرعة 100 ملغم / كلغ من خلات الرصاص لوحده بينت وجود زيادةُ معنويةُ (P <0.05)في عددِ التشوهات الكروموسومية خلال 28 يومِ خصوصاً الشكل الحلقي .

المعالجة بموانع التأكسد جرعة 0.5ملغم/كغم من السلينيوم و 0.2 مللتر من زيت الزيتون سبّبَ نقصان في عدد التشوهات الكروموسومية.

بينت النتائج ان اعطاء محلول خلات الرصاص لوحده سبّبَ نقصانَ (P <0.05 ملحوظ في مؤشر الانقسام الخيطي بينما اظهرت نتائج المعالجة بمضادات الاكسدة زيادةٍ في مؤشر الانقسام الخيطي في خلايا نُخاعِ عظم الفئرانِ خلال 28 يوم من زمن التجربةَ.

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