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Original scientific paper

INFLUENCE OF CADMIUM CHLORIDE ON THE FREQUENCY OF MICRONUCLEI IN DA AND AO RATS

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Heavy metal cadmium (Cd), a well–known environmental hazard, exerts a number of toxic and genotoxic effects. Experimental animals, Albino Oxford (AO) and Dark August (DA) rat strains, were treated intraperitoneally with three different concentrations of cadmium chloride ($CdCl_2$): 0.5, 1, and 2 mg $CdCl_2$ per kg of body weight, while the control animals received equal volume of sterile phosphate buffered saline. In this investigation individuals of both sexes aged 3, 6 and 12 month were used. Frequency of micronuclei formation was evaluated in polychromatic erythrocytes (PCEs), 24h hours after treatment. The results showed that that cadmium-cloride ($CdCl_2$) exibits the genotoxic effects causing an increase of the frequency of micronuclei depending on concentration, sex, age and strain.

Keywords: Albino Oxford, cadmium, Dark August, micronucleus, rats

INTRODUCTION

Heavy metal cadmium (Cd) is a well–known environmental hazard, which affects every organ system of the body. It is well documented that this heavy metal accumulates most highly in the mammalian liver and kidney. The chance of serious damage increases with continued exposure.

There are numerous examples which show that cadmium (Cd) is a highly toxic (DOI *et al*., 2006) and carcinogenic (HUFF *et al*., 2012) in humans (KAWAI *et al*., 2002), and animals (MARTIN

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et al., 2002). Cd potentially induce cytotoxic changes which inhibits erythropoiesis and cell differentiation (SINCLAIR and SINCLAIR, 1992).

Cd can damage DNA, inducing chromosome aberrations, micronuclei, sister chromatid exchanges (SCEs) and chromosomal loss in human and animals cell (JADHAV *et al*., 2006). Damaged DNA, possibly leading to the development of neoplasia by mutagenesis or clastogenesis, which may arise as a result of oxidative changes that may lead to oxidative stress. Oxidants produced during an electronic transport in mitochondria and peroxisomes may induce damage of various biomolecules including lipid peroxidation and DNA damage. Oxidative stress often occurs as a result of disrupted antioxidant systems in the body. Stimulated neutrophils and macrophages produce oxidants that cause cell damage *in vivo* and *in vitro* (BAGCHI *et al*., 2000). Cd-induced oxidative stress play a major role in aberrant gene expression (JOSEPH, 2009), DNA repair and apoptosis (DONG *et al*., 2001).

In vivo micronucleus test (MN test) detects whole chromosomes or chromosomal fragments incorporated in small nuclei (micronuclei) left outside the mitotic spindle during the cell division. Therefore, micronucleus test detects aneugenic (chromosome loss) and/or clastogenic effects (chromosome breakage) (LONČAR, 2007). MN test is convenient because the frequency of spontaneous micronuclei is small. Therefore, any increase in the frequency of MNi is attributed to the genotoxic effects of examined agent (SAVKOVIĆ *et al.*, 1990).

The incidence of micronuclated polychromatic erytrocytes (PCE) is particularly useful indicator of *in vivo* cytogenetic damage to bone marrow cells (SCHMID, 1975; SAVKOVIĆ *et al*., 1990).

We previously suggested that Cd induces micronuclei in bone marrow cells. Our previous study showed the ability of Cd to induce micronuclei formation in three months old AO rats (POPOVIĆ-BUBUJUK *et al*., 2013). Later, we included 6 and 12 month old AO rats of both sexes. The results showed that genotoxic effects of cadmium depended on age, sex and concentration (POPOVIĆ- BUBUJUK *et al*., 2014).

Numerous invstigations demonstrated that the metabolism of xenobiotics and their effcts on living organisms can vary greatly beetween species, strains, sex, age and individuals. Many of the variations seen with respect to metabolism of these compounds are due to genetic differences. Bearing this in mind, we compared possible differences in genotoxic effects of cadmium between two inbred laboratory rat strains - Dark August (DA) and Albino Oxford (AO). The genotoxic effects in these strains were evaluated by *in vivo* micronucleus assay.

MATERIALS AND METHODS

Chemicals

$CdCl_2$ (Serva Feinbiochemica GmbH, Germany) was dissolved in required amounts of sterile phosphate buffered saline to prepare three experimental concentrations: 0.5, 1, and 2 mg/kg of $CdCl_2$. Solutions were sterilised by filtration and stored at 4ºC before administration.

Cells were maintained in the RPMI-1640 medium (Sigma-Aldrich, USA) containing fetal calf serum (FCS) (ICN Flow, USA) at final concentration of 5% (v/v). FCS was previously inactivated for 30 min at 56°C. RPMI-1640 medium was also supplemented with HEPES (20 mM; Ivitrogen, USA), $NaHCO_3$ (0.85 g/l; Sigma-Aldrich, USA), L-glutamine (2 mM; ICN Flow, USA), and Gentamicin (8 µg/ml, ICN Flow, USA).

Ether (Betahem, Serbia) was used as anesthetic prior to sacrifice of animal by decapitation. Bone marrow smears on clean glass slides were fixed with absolute methanol (Sigma-Aldrich, USA). May-Grünwald and Giemsa stains (Sigma-Aldrich, USA) were used for erythrocyte staining and visualisation of MNi.

Experimental animals

All experiments were carried out with the consent of the Ethics Committee of Institute of Medical Research at the Military Medical Academy (MMA) in Belgrade.

Before the administration of $CdCl_2$, animals were set in groups of 5-7 individuals, and acclimated to the laboratory conditions for 5 days. The arrangement of the cages substantially minimized the occurrence of actual effects due to the cage placement.

Experimental animals were 3, 6 and 12 months old male and female DA rats, weighing 150-300 g, obtained from the Institute for Medical Research of the Military Medical Academy (MMA) in Belgrade, Serbia. The rats were kept at 25ºC and 12 h light: 12 h dark cycle. Animal were fed granulated food (Veterinary Institute Subotica JSC, Serbia) and supplied with water *ad libitum*.

Three different concentrations of $CdCl_2$ solution were administered in 5 ml aliquotes: 0.5, 1.0, and 2.0 mg/kg $CdCl_2$ (here in after referred to as the treated animals). The experimental design included two control groups, first of which received 0.5 ml sterile phosphate buffered saline and the other received no treatment whatsoever, used solely for the purpose of verifying the correctness of the method and absence of artifactual phenomena.

Experimental animals were anesthetized with ether and sacrificed 24 hours after the solution was administrated. For bone marrow preparations, femora were isolated, epiphyses cut off and bone marrow cells flushed out using a niddle and small amount of FCS. The cell suspension was centrifuged for 5 min at 1,000 rpm and sedimented cells resuspended. Fine bone marrow cell smears were prepared from the final cell suspension on grease-free clean glass slides. After air-drying (2-4 h) at the room temperature and fixing in absolute methanol (2-3 min), slides were stained using May-Grünwald-Giemsa staining method (SAVKOVIĆ, 1990).

The slide analysis was blinded and performed using a Nikon light microscope at magnification of 100×.

In vivo micronucleus test

The mammalian *in vivo* micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or mitotic apparatus of erythroblasts by analysis of erythrocytes sampled in bone marrow and/or peripheral blood cells of animals, usually rodents (OECD, 2014).

When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded, and MNi formed may remain in the otherwise anucleated cytoplasm. It is known that PCEs or immature erythrocytes contain RNA, therefore can be distinguished from mature normochromatic erythrocytes (NCEs), which are devoided of RNA (SAVKOVIĆ *et al.*, 1990).

Both PCEs and NCEs were determined for each animal by scoring 200 bone marrow erythrocytes, and one thousand of PCEs per animal were counted for the frequency of micronucleated cells (SCHMID, 1975; SAVKOVIĆ, 1990).

The number of MNi containing cells in 1,000 PCEs per animal, and the PCEs to NCEs ratio were analysed for every group of animals.

Positive control was included and aimed to produce MNi *in vivo* at the amount of 4 mg $CdCl_2$ per kg of body weight, which was expected to give a detectable increase over background. Therefore, these results are not presented in Table 1.

Statistical analysis

The results obtained in each experimental group of animals were compared with the controls and statistically analysed using Student's *t*-test and Z-test, according to which a result is considered statistically significant if the *p*-value is less than 0.05 ($P< 0.05$).

RESULTS

The frequencies of MNi in bone marrow PCEs were evaluated in 3, 6 and 12 months old male and female rats treated with different concentrations of the $CdCl_2$: 0.5, 1, and 2 mg/kg $CdCl_2$, are shown in Table 1 (DA) and Table 2 (AO). The frequency of MNi was calculated on 1,000 PCEs per animal. All the results are given with regard to the control group frequency of MNi in bone marrow PCEs. Control animals (untreated animals that received saline) served as the negative control group. There were no statistically significant differences in the frequencies of MNi between both control groups (untreated animals and those that received saline).

Table 1. The frequency of MN-RBC in polychromatic bone marrow of DA rats in different age groups and $CdCl_2$ concentrations. [a] $p<0.05$; [b] $p<0.01$ (Student t test in comparison to negative control).

Cd	DA males			DA females		
	3 mon.	6 mon.	12 mon.	3 mon.	6 mon.	12 mon.
Untreated animals	0,43 ± 0,53 n=7	0,43 ± 0,53 n=7	0,29± 0,49 n=7	0,29 ± 0,49 n=7	0,29± 0,49 n=7	0,86± 0,69 n=7
0 mg/kg b.w.	0,71 ± 0,49 n=7	0,71 ± 0,76 n=7	1,14 ± 1,07 n=7	1,71 ± 1,11 n=7	1,43 ± 0,98 n=7	1,29 ± 1,38 n=7
0,5 mg/kg b.w.	1,71 ± 0,76[a] n=7	1,57 ± 0,53 n=7	3,29 ± 1,25 n=7	2,14 ± 0,90 n=7	1,86 ± 1,21 n=7	2,14 ± 1,07 n=7
1 mg/kg b.w.	2,43 ± 0,79 [b] n=7	3,29 ± 0,76 [a] n=7	4,71 ± 1,50 [b] n=7	3,86 ± 0,90 [a] n=7	3,14 ± 0,69 [a] n=7	4,71 ± 1,11 [a] n=7
2 mg/kg b.w.	3,00 ± 0,58 [b] n=7	3,71 ± 0,49 [b] n=7	5,43 ± 1,14 [a] n=7	3,57 ± 0,79 [a] n=7	4,29 ± 1,11 [a] n=7	4,14 ± 1,35 [a] n=7

Table 2. The frequency of MN-RBC in polychromatic bone marrow of AO rats in different age groups and $CdCl_2$ concentrations. (These results were previously published in: Popović-Bubujuk et al., Genetika 2014, 46, No. 3, 1003-1012). [a]p<0.05; [c] p<0.001 (Student t test in comparison to negative control).

	AO males			AO females		
Cd	3 mon.	6 mon.	12 mon.	3 mon.	6 mon.	12 mon.
Untreated animals	0.57 ± 0.53 n=7	0.86 ± 1.07 n=7	0.71± 0.76 n=7	0.14 ± 0.38 n=7	0.86± 0.69 n=7	0.57± 0.79 n=7
0 mg/kg b.w.	0.57 ± 0.53 n=7	1.00 ± 1.00 n=7	0.86 ± 0.90 n=7	1.86 ± 0.38 n=7	1.14 ± 0.69 n=7	1.6 ± 0.89 n=5
0,5 mg/kg b.w.	2.86 ± 0.69 [c] n=7	2.14 ± 1.07 [a] n=7	1.00 ± 1.00 n=7	2.43 ± 0.53 n=7	2.29 ± 0.76 [a] n=7	3.25 ± 0.5 [a] n=5
1 mg/kg b.w.	3.86 ± 0.69 [c] n=7	3.57 ± 1.13 [c] n=7	2.86 ± 0.69 [a] n=7	3.43 ± 0.79 [c] n=7	3.14 ± 0.69 [c] n=7	4.5 ± 1 [c] n=5
2 mg/kg b.w.	4.43 ± 0.79 [c] n=7	4.29 ± 0.95 [c] n=7	3.57 ± 0.53 [c] n=7	4.5 ± 1.05 [c] n=7	3.86 ± 0.9 [c] n=7	4.5 ± 1.04 [c] n=5

There is a dose-dependent increase of MNi in all three age groups (3, 6, and 12 months) of DA males. The lowest dose of 0.5 mg/kg of $CdCl_2$, has led to an increase in the number of MNi, but on the border of statistical significance ($p < 0.05$), while the other two doses (1, and 2 mg $CdCl_2$ / kg of b. w.) led to a significant increase in MN ($p < 0.001$) in 3 month old males. The 6 and 12 month old males also had a dose-dependent significantly higher frequencies of MNi, but the levels of significance were different ($p < 0.05$: $p < 0.001$), indicating that different age groups of the same sex had significant increase in MNi, with different level of statistical significance.

The lowest dose of $CdCl_2$ (0.5mg / kg) has not led to a statistically significant in MN at all age groups of DA females. The remaining two doses led to statistically significant increase in MN in all age groups ($p < 0,05$), in comparison to the control group. In females, there is no age-dependence of the mutagenic effects of the $CdCl_2$.

By using Z-test we compared the MN values obtained various age groups of the same sex, and values between different sexes of the same age group, for all the applied doses (Table 3 and 4).

Table 3. Comparison of the frequencies of MN-RBC in bone marrow of DA rats between different age groups of the same sex and treatment of various doses of Cd, $|Zo| > u\alpha/2$ denotes statistical significance, while $|Zo| < u\alpha/2$ absence of statistical difference.

	DA males			DA females		
Cd	**3/6mon.**	**3/12 mon.**	**6/12 mon.**	**3/12 mon.**	**3/12 mon.**	**6/12 mon.**
0,5 mg/kg b.w.	n.s.	2,86>1,96	2,03>1,96	n.s.	n.s.	n.s.
1 mg/kg b.w.	2,08>1,96	2,56>1,96	3,98>1,96	n.s.	n.s.	3,18 >1,96
2 mg/kg b.w.	2,47>1,96	4,22>1,96	3,07>1,96	n.s.	n.s.	n.s.

Table 4. Comparing the frequency of MN in bone marrow of DA rats of the same age group between different genders and different doses of Cd treatment, Zo|> uα/2 denotes statistical significance, while |Zo|< uα/2 absence of statistical difference.

	DA males-females		
Cd	**3mon.**	**6 mon.**	**12 mon.**
0,5 mg/kg b.w.	n.s.	n.s.	n.s.
1 mg/kg b.w.	3,16 >1,96	n.s.	n.s.
2 mg/kg b.w.	n.s.	n.s.	n.s.

Comparisons between different genders within the same age shows that the most profound differences are between DA males and females at the age of 3 months.

The 12 months old males and females react similarly to the lowest and highest applied dose, while at the dose of 1 mg/kg $CdCl_2$ there were a varying degrees of significance ($p < 0.001$; $p < 0.05$: males and females, respectively). MN test showed that males were more sensitive than females with more clear dose- and age-dependent effects of Cd.

In Table 5 we presented the frequency of MN in bone marrow of the DA – AO rats between the same age, same gender, different strains and treatment of different doses of Cd.

Table 5. Comparing the frequency of MN-RBC in bone marrow of AO-DA rats of the same age groupbetween different strains and treatment of variousdoses of Cd, where is |Zo|> uα/2 has a statistical difference, and when |Zo|< uα/2 there is no statistical difference.

	DA/AO males			DA/AO females		
Cd	**3 mon.**	**6 mon.**	**12 mon.**	**3 mon.**	**6 mon.**	**12 mon.**
0,5 mg/kg b.w.	2,95 >1,96	n.s.	3,82>1,96	n.s.	n.s.	2,41>1,96
1 mg/kg b.w.	3,56 >1,96	n.s.	2,98 >1,96	n.s.	n.s.	n.s.
2 mg/kg b.w.	3,86 >1,96	n.s.	3,26 >1,96	n.s.	n.s.	n.s.

Comparisons of the same age groups of DA and AO males shows significant differences between 3 and 12 month old animals for all three applied doses. When females are concerned, a statistically significant difference was found only at 12 months old group that received the lowest dose applied. Genotypic differences, ie. differences between strains are more common in males.

DISCUSSION

Our previous investigations demonstrated genotoxic effects of $CdCl_2$ administered intraperitoneally into three age groups (3, 6, 12 months) at three concentrations (0,5, 1.0, and 2.0 mg $CdCl_2$ per kg body weight), and both sexes of AO rats (POPOVIĆ–BUBUJUK *et al*., 2013;

POPOVIĆ–BUBUJUK *et al*., 2014). In this study, rat bone marrow was used for testing the clastogenic or aneugenic effects of Cd. Genetic damage expressed as micronuclei following $CdCl_2$ administration was assessed in genetically pure *DarkAugust* (DA) rats. MN test was applied in order to investigate, in addition to dose dependence, age and sex differences in response to the effect of the Cd, as well as possible role of genetic differences between strains. In both strains, both sexes, all three age groups had a positive dose–dependent increase in the number of micronuclei. There were no major discrepancies in the number of micronuclei and among the control animals (untreated and those that received saline) in all age groups (3,6,12 month) and both sexes of either strain. MN test showed that there are differences among age groups and both sexes of rats.

There are already literature data which show that there are gender differences in the accumulation, distribution and detoxication of Cd (SWIERGOSZ-KOWALEWSKA, 2001). Cd absorption and biohemical conversion are more profound in females. Investigations of GUBRELAY *et al*. (2004) showed that females are more prone to acute effects of Cd than males. Gender differences are more pronounced in the neonatal period. In neonatal *Sprague – Dowley* rats, $CdCl_2$ reduce body weight, kidney and spleen weight in females but not in males, unlike in adult rats (PILLET *et al*., 2005). In adults gender differences are most clearly seen in the liver and kidney. Female liver contains greater amount of Cd in comparison to males (BAKER *et al*., 2003).

Greater variations in concentration of Cd in renal tissue were present more in females than in males in different strains of rats (SHAIKH *et al*., 1993). ZELLWEGER *et al*. (1997) found that differences in immune response between male and female C3H/HEN rats are reflected through reduced spleen cells release of IL-2 and IL-3 in males. That was the reason why females were more sensitive to Cd in comparison to males. The similar results were obtained in humans. Immune system of women is more sensitive than the immune system of men (BUTTER, 2006). For example, ”Itai-itai” disease caused by intoxication Cd is more common in females than in males (HAMADA *et al*., 1991).

Gender-depended differences of the effects of Cd probably result from the action of hormones. Sex hormones act directly on the Cd (BUTTER, 2006). Cd can also mimic estrogen (JOHNSON *et al*., 2003). Progesteron-activated calcium chanals are involved in the hepatotoxcity of Cd (BAKER *et al*., 2003). The similar case is with heart of adult female rats in which the amount of inflammatory cytokines is reduced (WANG *et al*., 2004). Females take and retain a large amounts of Cd in the liver, but have a greater ability of detoxification because they contain more kidney metalothionein (MT) than males (KLAASSEN *et al*.,1999).The amount of MT also plays an important role in the pulmonary inflammation (ČANČAR,1998). As a result, the frequency of MN in polychromatic red blood cells of bone marrow DA strain differs significantly by sex.

As for the age groups, the obtained results suggest that the MN frequency of variability of the analysed samples is large due to a complex factor - age (VICRAM *et al*., 2007). In chick embryo, PATTEN (1920) reported earlier that the spleen is not functional organ at 11^{th} day of the development. So the MN formed in erythrocytes cell accumulate more in blood since spleen is yet not functional to clear them from blood. With Cd was capable of inducing MN in erythrocytes. This effect was further confirmed by PCE / NCE ratio which indicates hemotopoietic cell proliferation rate to infer the hampered hemotopoiesis or cytotoxicity. Cd-treated chick embryos show extensive damage to chromosomes in blood cells (BAI *et al*., 2014). Mammals are also sensitive to toxic metals from the environment especially in the early stages of

development (PILLET *et al*., 2005). Interestingly, cells of pregnant rat females are more sensitive to clastogenic effects of Cd and various metals after *in vivo* and *in vitro* treatment. (YUAN *et al*., 2013). Cd induces fetal malformations, as well as a significant increase in the quantity of micronucleated normochromatic erythrocytes (MNNE) in blood cells of the pregnant ICR mice and their fetuses (ARGUELLES–VALAZGUEZ *et al*., 2013).

In 3 months old male Wistar rats Cd caused oxidative damage that resulted in reduction in number of red blood cell, concentrations of hemoglobin and increased ALT, the AST, LP, GSH, as well as the increased glycaemia (WERSHANA, 2001). In DA rats of the same age increased total white blood cell count was found that is caused by increasing the number of granulocytes in all applied doses, and histological evaluation of lungs after giving intraperitoneal $CdCl_2$ showed a toxicity of Cd, to the presence of inflammatory processes of varying intensity (ČANČAR, 1998). All has proved to increase the level of antioxidant enzymes, including SOD, glutathione peroxidase and reduction glutathione (ABDEIL–DAM *et al*., 2013).

Among the erythrocytes of the bone marrow, polychromatic (immature) erythrocytes showed a significant age-dependent increase in the frequency of micronuclei in 1-20 month old Swiss albino mice. The increase in MN frequency was less pronounced in normochromatic (mature) erythrocytes. These results show that there is an accumulation of genetic damage in bone marrow and DNA damage in peripheral blood of mice during aging and that females show more alternations than males (BHILLWADE *et al*., 2014). Also, there is an increase of Cd accumulation with age, and the frequency of MNi is also increasing with aging (MILOŠEVIĆ–ĐORĐEVIĆ *et al*., 2004). High mortality of 75% occurred in the aged (24month-old) group following Cd administration, indicating age to be an important sensing factor in the toxic hazard of heavy metal exposure (WORMSER and NIR, 1988). The age difference is more pronounced in male DA rats (ČANČAR, 1998).

In addition to sex and age- dependent differences we also observed differences between strains. So there are strains of mice that are sensitive (C3H, BALB/C, C57BL/6) and those who are not (DBA, CBA/J, CD1). The similar case is with rats – Fisher 344 males Similar are more susceptible to chemical damage of the liver than Sprague-Dawley strain (KUESTER *et al*., 2002). In addition, SHIMADA *et al*. (2009) reported that Wistar – Imamishi rats are highly resistant to Cd-induced lethality and hepatotoxicity in contrast to Fisher 344 rats. They examined possible strain-related differences in Cd-induced testicular toxicity between inbred Wistar–Imamishi and Fisher 344 rats. Namely, Fisher 344 rats treated with a single dose of 0,5, 1 and 2 mg/kg of $CdCl_2$ had severe testicular hemorrhage and increased hemoglobin content, which was not the case with Wistar–Imamishi rats. After the treatment with 2mg/kg of $CdCl_2$ the testicular Cd content was significantly lower in Wistar – Imamishi rats than in the Fisher 344 rats indicating a toxokinetic differences between strains. The remarkable resistance to Cd-induced testicular toxicity in Wistar–Imamishi rats is associated with lower testicular accumulation of Cd. The differences between strains exist in DA and AO when the liver, kidney and lugs were examined (ČANČAR, 1998). The answer may be in the level of TNF activity in the circulation in terms of a generalised inflammatory response (KATARANOVSKI *et al*., 2003), or can be explained by differences in the amount of methallothioneins (KUESTER *et al*., 2002). Cd-competes with Zn transport systems (SHIMADA *et al*., 2009) which may be a reflection of differences between genotype groups of highly inbred animals, as shown in our results.

Effects are dose-dependent for all groups treated with Cd. Frequency of micronucleated erythrocytes increase with increased doses of Cd and time interval (BAI *et al*., 2014). When it

comes to high doses of $CdCl_2$ changes are manifested not only in MNi but there is damage to mature red blood cells that are seen in the red blood cells, or on its membrane (SINDAR and SINCLAR, 1992). All exposures resulted in a significant increase in frequency of abnormal erythrocytes. Anomalies induce chromatin condensation at the nucleus border, nuclear malformation being the most frequent (WITESKA *et al*., 2011). Micronuclei formation in humans is associated with various medical conditions (LUZHNA *et al*., 2013). Human contact significantly increased chromosomal aberrations and sister chromatid exchange in all age groups (ABRAHIM *et al*., 2011).

CONCLUSIONS

In these experiments, we showed that $CdCl_2$ treatment significantly increased the frequency of micronuclei in polychromatic erythrocytes (PCE) of bone marrow of DA and AO rats depending on concentration, gender, age and strain.

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UTICAJ KADMIJUM HLORIDA NA UČESTALOST MIKRONUKLEUSA KOD AO I DA PACOVA

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Izvod

Teški metal kadmijum (Cd), predstavlja opasnost iz životne sredine sa brojnim toksičnim i genotoksičnim efektima. Eksperimentalne životinje Albino Oxford (AO) i Dark August (DA) sojevi pacova tretirani su intraperitonealno sa tri različite koncentracije kadmijum hlorida ($CdCl_2$): 0.5, 1 i 2 mg $CdCl_2$ po kg telesne mase, dok su kontrolne životinje dobijale istu zapreminu sterilnog fosfatnog pufera. U ovim istraživanjima korišćene su jedinke oba pola stare 3, 6 i 12 meseci. Učestalost stvaranja mikronukleusa evaluirana je na polihromatskim eritrocitima (PCE) nakon tretmana od 24 h. Rezultati su pokazali da je kadmijum hlorid ($CdCl_2$) ispoljio genotoksične efekte prouzrokujući povaćanu učestalost mikronukleusa zavisno od koncentracije, pola, uzrasta i soja ispitivanih pacova.

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