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Genotoxicity of Cobalt Oxide Nanoparticle in Rat Bone Marrow Single Cell Gel Electrophoresis-Micronucleus Test

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ABSTRACT

Nanoparticles can be synthesized in different shapes and used in different fields of industry due to their physicochemical properties. The adult male Wistar rats were exposed to CoONPs (≤50 nm nanopowder) through gavage administration at 1, 5, 10 and 50 mg/kg body weight every day for 28 days. In this study genotoxicity was tested by using the single cell gel electrophoresis (COMET) assay and micronucleus (MN) test systems in rat bone marrow. CoOnp treatment significantly induced the frequency of micronucleus and DNA damage ($p<0.05$). The decrease in ratio of polychromatic erythrocytes to normochromatic erythrocytes is significant ($p<0.05$). These findings have important implications for understanding the potential health effects of CoOnp exposure.

Keywords: Cobalt Oxide Nanoparticle; Bone Marrow; Polychromatic erythrocyte; Comet Assay; Micronucleus;

1. INTRODUCTION

Nanotechnology has a great attention and a significant progress in many fields of science such as in medicine and in the production of commonly used materials. Nanoparticles (NPs) in different shapes such as nanotubes, nanocage, nanorods, etc., can be synthesized and used in different fields of industry due to their unique physicochemical properties (Salahdin et al. 2022). Recently, there has been a dramatic increase in the use of nanoparticles not only in industrial sectors, but also in pharmaceutical/medical applications and food production/packaging (Kuzma and Priest 2010,Fu, et al. 2004). Because of their properties, such as high surfaceto-volume ratios and reactivity, may affect their toxicological characteristics, as compared to nonNM counterparts (Kuzma and Priest 2010). Despite the various benefits of nanoparticles, less is known about their risks to human health and the environment. Humans can be exposed to NPs through inhalation, topical application, or oral intake. Oral exposure may be significant because of the use of NPs as food additives and in packaging materials. Ingestion through the food chain, due to environmental contamination, can also occur. Due to their small size, they can evade the immune system, enter circulatory apparatus, and reach organs. Being smaller than cells, NPs can penetrate cell membranes, enter organelles, and disturb cell physiology, leading to cytotoxicity and genotoxicity. A large amount of data including in vitro cell studies, in vivo studies in animals and humans, epidemiological studies, and occupational health

studies report on nanoparticle toxicity. Most information on NP toxicity derives from in vitro or in vivo studies with short-term or acute administration via intravenous (iv), intraperitoneal (ip), or inhalation routes. Limited information concerning oral long-term exposure is available, although only such studies take into account tissue accumulation and persistence, which may be key factors for the induction of organ toxicity. (IARC 2006, EFSA 2018) also, NPs are present in bulk material as pollutants due to natural events or anthropogenic activity (Donaldson, et al. 2004). Consequently, humans are exposed to nanoparticles through dermal absorption, ingestion and inhalation due to occupational and/or environmental conditions. NPs can affect macromolecules in cell and play a role in morphological/physiological changes in the exposed organ or on the lung, liver, kidney and gastrointestinal and nervous systems (Brooking et al, 2001, Barillet 2010, Arora, et al. 2012). In studies performed with many nanoparticles, it has been shown that nanoparticles have genotoxic, cytotoxic and apoptotic effects in various cells under in vitro culture conditions and in vivo (Battal, et al. 2015, Alarifi, et al. 2013, Esamaldeen Ebrahim Mohamed, et al. 2021, Carmona et al. 2015).

Among nanoscale materials, metal NP are already commercially available for several applications in the fields of biology, medicine and pharmacology. One of the most interesting chemical elements used as NP for biomedical applications is cobalt (Co); it can be produced as Cobalt oxide (CoO), as an organometal compound or as a biopolymer). (Wang, et al 2015). In spite of its physiological role as a cofactor of vitamin B12, Co cannot be regarded only as an essential element. Co is widely used in the industry because of its high temperature resistance, corrosion resistance, and wonderful magnetic properties. $Co2+$ ions are genotoxic, and several forms of cobalt are carcinogenic in experimental animals. Upon inhalation exposure, the respiratory system is the main target organ of cobalt in humans (asthma, fibrosing alveolitis). The risk of fibrosing alveolitis (hard metal disease) and lung cancer is specific of the hard metal industry, where workers are exposed to cobalt metal mixed with tungsten carbide particles. Other target organs include the erythropoietic system, the myocardium, the thyroid gland, and the nervous system. In patients with cobalt alloy implants, especially metal-on-metal hip prostheses, endogenous exposure to cobalt can result in local and/or systemic toxicity. The reproductive toxicity of cobalt compounds is not well documented (Balram and Zhang 2017). Cobalt nanoparticles (Nano-Co), as an important nanomaterial, have expanded its applications. For example, as a magnetic material, Nano-Co has been used in nuclear magnetic resonance contrast agents, military invisible materials, ceramics, biomedical fields (Wolf, et al. 2019, Pichéet al. 2019). Many previous studies showed that exposure to Nano-Co caused oxidative stress and severe, lung inflammation and fibrosis, which were strongly linked to pulmonary toxicity (Zhang, et al 1998, Wan, et al. 2017). It has been reported that cobalt oxide (CoO) NPs activate caspase reactions and trigger cell death by increasing ROS in vitro and in vivo (Chattopadhyay, et al. 2015). There are many test models that make it possible to examine the genotoxic effect. The mammalian in vivo micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents. Micronucleus test method has been used to evaluate the potential effects of chemicals for decades in many researches in in vitro and in vivo studies in different living systems (Çelik, et al. 2003, Çelik, et al. 2005, Çelik and Kanık, 2006) for cyto/genotoxicity. The single cell gel electrophoresis assay (SCGE) or comet assay provides the opportunity evaluating the DNA damage responses of individual cells exposed to DNA damaging agents and visualizes DNA migration within nucleus embedded in agarose. (Singh, et al. 1988) The depth of migration is related to the greatness of single strand breaks in damaged DNA [Narendra 2000, Çavaş 2011, O'Donoghue, et al. 2021). Cyto- and genotoxicity are commonly studied endpoints, and both are relevant, but it is important to realize that nanomaterials may perturb biological functions in the absence of overt cell death. Therefore, the aim of the present study was to

explore the in vivo genotoxic activity associated with the exposure of cobalt oxide NPs using two different genetic endpoints (comet assay and Micronucleus test) in bone marrow cells of Wistar rats.

2. MATERIAL AND METHODS

2.1. Chemicals

CoOnp (<50 nm- TEM) nanopowder was commercially obtained from Sigma-Aldrish Chemical (Lot# MKCF8353 99.5 %, trace metals). Other chemicals or solvents used in this study were of cell culture, HPLC, or analytical grade. Roswell Park Memorial Institute (RPMI) medium, phosphate buffer solution (PBS), normal melting agarose (NMA), Trisma base, Triton X-100, ethylenediaminetetraacetic acid (EDTA), ethidium bromide (EtBr) were purchased from Sigma. Sodium chloride (NaCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) was purchased from Merck. Low melting agarose (LMA), Fetal Calf Serum were purchased from Bioshop, Biochrom AG, respectively.

2.2. Dose selection

The dose selection was determined by considering the study in which the LD_{50} dose of nanoparticles (Shaikh, et al. 2015).

2.3. Animal and experimental design

In this study, healthy adult female Swiss albino rats (Wistar rat) [12 months old and average body weight (body w.t.) of 380-400 g] were used. Rats were obtained from the Experimental Animal Center, University of Mersin, Turkey. **This study was approved by the ethics committee of the University of Animal Studies of Mersin University (23/09/2019-2019/24).** The rats were randomly selected and housed in polycarbonate boxes (six rats per box/six group) with steelwire tops and rice husk bedding. They were maintained with 12 h light /dark cycle in an atmosphere of 19-22^{0}C temperatures and 55–65% humidity with free access to pelleted feed and tap water. The animals were allowed to acclimate for 12 day before experimental study. Therefore, this study was carried out with five different dietary programs at a dose of 24 hours for 28 days. Positive control group was treated with mitomycin C (2mg/kg). Rats were anesthetized with Ketalar (Ketamine-HCl (40 mg/kg) Pfizer, +Xylazine HCl, (10 mg/kg) (Rompun flakon, Bayer), 4 h after the last treatment and sacrificed. The same rats were used for both Comet assay and Micronucleus test analysis. The same dose regimens were used for both Comet analysis and Micronucleus test.

The rats were treated by gavage with

(i) The untreated control rats were treated identically with equal volumes of normal saline only via gavage throughout the study.

(ii) 1 mg/kg 50 nm cobalt oxide nanoparticle solution (0.55) dissolved in sunflower oil

(iii) 5 mg/kg 50 nm cobalt oxide nanoparticle solution (0.5 mL) dissolved in sunflower oil

(iv) 10 mg/kg 50 nm cobalt oxide nanoparticle solution (0.5 mL) dissolved in sunflower oil

(v) 50 mg/kg 50 nm cobalt oxide nanoparticle solution (0.5 mL) dissolved in sunflower oil

(vi) In this study, a single dose of MMC (2 mg/kg, i.p.) was administered since positive controls may be administered by a different route and treatment schedule than the test agent. (Hayashi, et al. 1994).

2.4. Comet assay in bone marrow

The one femurs were removed and bone marrow cells were flushed from the femur into 1 mL of fetal bovine serum (FBS). Isolated bone marrow cell suspension was washed two times with RPMI supplemented with 10% FBS. Part of the isolated cells was used directly for the comet assay. Comet assay was performed under alkaline conditions according to the method of Singh et al. (1988) with slight modifications. One side frosted microscopic slides were covered by a thin layer of 0.5% normal melting agarose (NMA) dissolved in Ca^{2+} and Mg^{2+} free phosphate buffer saline (PBS) at about 50^0C. Eppendorf tubes were placed in heatwater bath at 40^0 C. One hundred microliters of bone marrow suspension were diluted with 1 mL of PBS in eppendorf tube. Then 35 µl mixtures were mixed with 265 µL of LMA (0.5%). One hundred microliters of this mixture was spreaded on NMA-coated slides using micropipette and immediately was covered with coverslip. Slides were preserved in refrigerators at +4 C for 17 min. The coverslips were slowly removed from top of slides. Then slides were placed in chalets including lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mMTris, 1% Triton X100, pH 10) for 2 hours at 40 C^0 and preserved for 1.5 h in refrigerator in dark. Slides were washed with chilled distilled water and placed on a horizontal gel electrophoresis unit filled with fresh electrophoretic buffer (300 mM NaOH + 1 mM EDTA) to allow DNA unwinding before electrophoresis for 20 min. Electrophoresis was conducted at 20^0C using 25 V and 185 mA for 20 min. The above steps was carried out in dark to avoid DNA damage. After electrophoresis, slides were washed with chilled distilled water and placed in neutralizing buffer for 15 min. Then again slides were washed with chilled distilled water and placed in chilled absolute ethanol for 10 min. The slides was stained with ethidium bromide (0,1 mg/mL, 1:4) and were examined with a fluorescent microscope (BX 51, Olympus, Japan) for analysis.

2.4.1. Scoring of comet images

Comet images were analyzed according to Collins et al., (1997) One hundred comet images were scored for each treatment-animal by two scorers (ÇELİK and SİNACİ.) visually under fluorescence microscopy (BX51 Olympus). An intensity score from class 0 (undamaged) to class IV (ultra high damage) was assigned to each cell. The observation method is blindly blocked that the observers have no knowledge of the identity of the slide.

$$AU = \sum_{i=0}^{4} i \times N_i$$

Ni: is the number of scored cells in i level, i is the level of DNA damage (0, 1, II, III, and IV).

- Genetic damage index: GDI: [(0xType 0) + (1xType I) +(2xType II) +(3xType III) +(4xType IV)]

GDI (AU values) indicating the comet assay scores show levels of UD (undamaged, 0), Type 1 (low damaged, 1), Type II (moderate damaged, II), Type III (high damaged, III), and Type IV (ultra high damaged, IV).

- Damaged cell percentage (DCP): Type II+Type III+Type IV (Sinaci, C., et al. 2023).

2.5. Bone marrow micronucleus test

The bone marrow was evacuated from both femora using 1 mL of fetal calf serum and centrifuged at 4000 rpm for 10 min and the supernatant was allocated. Bone marrow were smeared on clean microscope slides, air dried, fixed in methanol and stained with acridine orange (125 µg/mL in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluorescence microscope (Olympus BX51) using a 40X objective. The frequency of micronucleated erythrocytes in femoral bone marrow

was evaluated according to the procedure of Schmid (1973), with the slight modifications of Agarwal and Chauhan, (1993).

2.5.1.Scoring of micronucleus frequency

The proportion of immature among total (immature + mature) erythrocytes is determined for each animal by counting a total of at least 200 erythrocytes for bone marrow (Hayashi et al., 1994). Number of Micronucleated Polychromatic Erythrocytes (MNPCEs) was determined using 2000 PCEs per animal. Briefly, immature erythrocytes, i.e. PCEs are characterized by red orange colors, mature erythrocytes are characterized by their green color and micronucleus as yellowish green. (Schmid, 1973, Agarwal and Chauhan, 1993)

2.6. Statistical examination

Data were compared by one-way variance analysis. All statistical analysis was performed using the SPSS for Windows 21.0 package program. Multiple comparisons were performed by LSD (Least Singificat Difference) test. Statistical significance was considered as $p<0.05$. Pearson Correlation test was used to determined the relationship among parameters.

3. RESULTS

The raw data of micronucleus, the ratio of polychromatic erythrocytes to normochromatic erythrocytes and the comet parameters, GDI and DCP, in bone marrow of negative/ positive control are shown in Table 1.

Table 1. Results of Comet assay scores (genetic damage index and damaged cell percentage) and Micronucleus frequency /polychromatic erythrocytes number in bone marrow of rats sub-chronic gavage- exposure to Cobalt oxide nanoparticles (50 nm)

Groups	Rat	0	1	2	3	4	AU(GDI)	DCP	MN/2000 PCE	PCE(total erythrocytes)
Negative Control	1	60	34	6	0	0	48	6	2	100
	2	68	26	6	0	0	38	6	1	101
	3	65	28	5	2	0	44	7	2	102
	4	74	20	6	0	0	32	6	2	100
	5	67	25	7	0	0	39	7	2	102
	6	68	25	6	1	0	30	7	2	103
Group 2 (1mg/kg) CoO nnp)	1	27	7	14	9	43	234	66	2	85
	2	30	6	17	9	42	235	68	2	85
	3	26	9	15	6	44	233	65	3	86
	4	28	6	15	9	42	231	66	2	85
	5	26	11	13	8	42	223	63	2	82
	6	27	8	14	8	43	232	65	3	85
Group 3 (5mg/kg) CoO nnp)	1	30	12	8	11	40	231	59	4	72
	2	28	10	12	8	42	226	62	5	75
	3	32	8	5	10	45	228	60	6	74
	4	24	6	15	13	42	243	70	4	75
	5	23	10	17	5	45	239	67	5	75
	6	23	12	13	9	43	237	65	6	74
Group 4 (10mg/kg) CoO nnp)	1	20	15	10	15	40	240	65	5	75
	2	22	14	14	8	42	234	64	5	68
	3	20	10	13	12	45	252	70	6	75
	4	20	8	18	12	42	248	72	6	68
	5	19	14	17	15	35	223	67	5	69

	6	12	14	17	14	43	262	74	5	69
Group 5 (50mg/kg) CoO nnp)	1	22	11	11	15	41	242	67	6	72
	2	25	12	12	13	38	227	65	6	75
	3	26	10	11	13	40	211	64	5	72
	4	20	10	11	19	40	249	70	6	69
	5	19	13	11	18	39	245	68	6	75
	6	21	12	14	12	41	240	67	6	75
Postive Kontrol 2mg/kg (MMC) Mitomycin C	1	2	3	11	28	56	333	95	12	66
	2	0	2	12	28	58	344	98	15	60
	3	2	8	10	21	59	327	90	15	62
	4	3	3	14	23	57	328	94	16	60
	5	2	3	10	25	60	338	95	18	61
	6	2	6	12	25	55	325	92	12	62

Table 2 represents mean frequencies of micronucleus, the ratio of polychromatic erythrocytes to normochromatic erythrocytes and the comet parameters, GDI and DCP, of negative/ positive control and groups treated with dose of 1 mg/kg, 5 mg/kg, 10 mg/kg, 50 mg/kg of CoOnp in rat bone marrow.

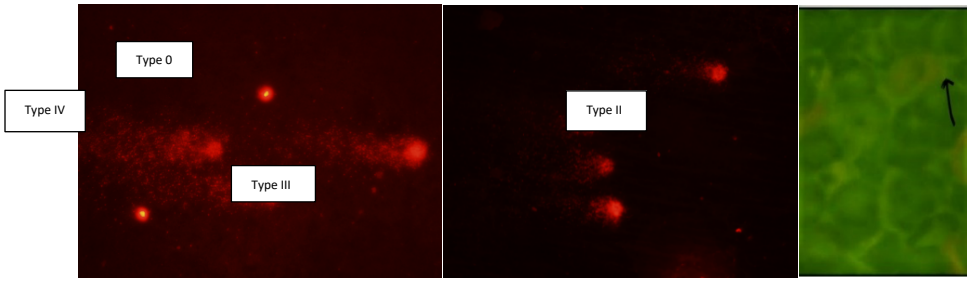
Table 2: Comet assay scores (genetic damage index and damaged cell percentage) and Micronucleus frequency /polychromatic erythrocytes number in bone marrow of rats sub-chronic gavage- exposure to Cobalt oxide nanoparticles (mean ± S.E.)

Groups	**Genetic damage index (GDI)** **Mean ± S.E**	**Damaged cell percentage (DCP)** **Mean ± S.E**	**MN/2000PCE** **Mean ± S.E**	**PCE/200 total erythrocytes)** **Mean ± S.E**
Negative control	38.5±2.80	6.50±0.22	1.83±0,16	101.3±0.49
Cobalt oxide nnps (50 nm) 1 mg/kg	231.3±1.76***	65.5±0.67***	2.33±0,21	84.6±0.55***
5 mg/kg	234.0±2.73***	63.8±1.74***	5,00±0.36***	74.1±0.47***
10 mg/kg	243.1±5.64***	68.6±1.62***	5.33±0.21***	70.6±1.38***
50 mg/kg	235.6±5.79***	66.8±0.87***	5.83±0.16***	73.0±1.00***
Positive control (MMC-2mg/kg)	332.5±2.99***	94.0±1.12***	14.6±0.95***	61.8±0.90***

***p<0.001 compared with negative control

DCP: Damaged cell percentage; GDI: Genetic damage index; MN:Micronucleus; PCE: Polychromatic erythrocyte

Figure 1 presents comet assay views (a-b) and the micronucleus in polychromatic erythrocytes (c).



(a) (b) (c)

Figure 1: Ethidium bromide stained-comet views (a-b) and acridine orange stained-micronucleated polychromatic erythrocytes (c) of rat exposed to CoNP (50 nm). Arrow indicates the MN

In this study, statistical analysis indicated that there is a significant difference between groups and within groups for MN frequency, PCE and Comet parameters, GDI and DCP values. It was observed that there is a dose-related statistically significant increase in the MN frequency on rat bone marrow exposed to Cobalt oxide nanoparticle (50 nm) (Table 2). MN frequency reached to 2.33±0.21, 5.00±0.36, 5.33±0.2, 5.83±0.16 in groups exposed to Cobalt oxide nanoparticle (50 nm), 1 mg/kg, 5 mg/kg, 10 mg/kg and 50 mg/mL, respectively and to 1.83±0.16, 14.6±0.95 in negative, positive control group. The results of the MNPCE analysis indicate that there was a difference between negative control and all the doses of Cobalt oxide nanoparticle ($p<0.001$) except of 1m/kg dose. It is found that CoOnp (50 nm) induced the DNA damage level in both GDI and DCP parameters in alkaline comet assay analysis. The values of GDI and DCP reached to, 231.3±1.76, 234.0±2.73, 243.1±5.64, 235.6±5.79 and, 65.5±0.67, 63.8±1.74, 68.6±1.62, 66.8±0.87 in group exposed to CoOnp (50 nm), 1 mg/kg, 5 mg/kg, 10 mg/kg and 50 mg/kg and to 38.5±2.80 and 6.50±0.22, 94.0±1.12 in negative, positive control group, respectively. MMC administered as positive control induced a statistically significant increase in the mean number micronucleus and DCP and GDI values ($p<0.001$) compared with negative control. There is a significant difference between all the doses of CoOnp (50 nm), for PCE/total erythrocytes ($p<0.001$). Table 3 represents Pearson correlation results between, comet analysis data and micronucleus values. The existence of a relationship between the DCP and GDI values, which are the comet analysis parameters, and the Micronucleus frequency values show that the two test methods support each other.

Table 3. Pearson correlation results between, comet analysis data and micronucleus values.

	GDI	DCP	MN	PCE
GDI	1	,997****	,722***	-,930****
DCP	,997****	1	,708***	-,927****
MN	,722***	,708***	1	-,770***
PCE	-,930****	-,927****	-,770***	1

***** high level of correlation, ****ultra high level of correlation**

DCP: Damaged cell percentage; GDI: Genetic damage index; MN:Micronucleus; PCE: Polychromatic erythrocyte

4. DISCUSSION

Nanoparticles, which form the basis of nanotechnology, have started to take a place in many areas of our lives due to their many physicochemical properties such as size, shape, surface area, surface charge and solubility that they have newly acquired thanks to their nano dimensions. Nanoparticles are used in many fields such as electronics, textile, paint industry, military materials, food, biomedical and automotive sectors due to their superior properties. With the widespread production and use of nanoparticles, the increase in human exposure on the one hand and the increase in their waste on the other hand pose direct or indirect health risks to both humans and all living things in the ecosystem. For all these reasons, it is of great importance to investigate the toxic, especially genotoxic effects of nanoparticles. Fundamental similarities in cell structure and biochemistry between animals and humans provide a valid basis for prediction of the likely effects of chemicals on human populations (Meyers, 1993). In this study, we investigated two mechanisms of genotoxic action often described for NP, i.e., chromosomal damage due to interference with the mitotic spindle and DNA damage, bone marrow polychromatic erythrocytes of rats exposed to CoOnp. Chromosomal damage can be observed either in interphase cells as MN or in metaphase cells as chomosome aberrations. We

already reported an increase in the number of micronucleated polychromatic erythrocyte cells induced by CoOnp in rat bone marrow. Studies usually involve introducing nanoparticles to cells in vitro so that the cells internalise the nanoparticles, resulting in a dose of nanoparticle per cell. It has generally been established that toxicity is again dose-dependent, with high doses eliciting negative effects that reduce cell viability such as cell membrane disruption (Rajiv, et al. 2016), altered cell cycles (Wu, and Sun, 2011), reduced motility (Coreman Berman, et al.2013) and genotoxicity (Valdiglesias, et al. 2015) to note a few.

The data on the genotoxic effects of nanoparticles are rather controversial as different studies have reported different results depending on the test system, administration route or organism used in the experiments. Most toxicological studies on nanoparticles and nanomaterials have been performed in vertebrates or laboratory rodents and limited data are available for other species. The current study was focused on genotoxic and oxidative DNA damage effects of Co-Nps in bone marrow cells of Wistar rats. DNA damage and micronucleus formation were used as genetic endpoints. [Çelik et al. 2003, Çelik and Kanık 2006, Çelik et al. 2013). Recent studies on the genotoxic effects of nanoparticles on mammals cells have revealed that these particles can cause DNA damage and changes in cell kinetic parameters. It has been shown that many nanoparticles of different concentrations and different sizes cause chromosomal abnormalities, sister chromatid exchange, micronucleus and damage to DNA. (Antonoglou, et al. 2019). Depending on the developments in nanotechnology and the widespread use of nanoparticles in this technology, the exposure of both humans and all other living things to these particles has started to increase due to the production, use and waste of nanoparticles. Therefore, in recent years, concerns about the toxic effects of these particles, especially their genotoxic effects, have begun to increase (Assadian, et al. 2018, Giorgetti, 2019). The genotoxic effects of nanoparticles are investigated with various test techniques, primarily, in vitro on human lymphocytes (Battal, et al. 2015), in various cell lines such as fibroblast (L929) TK6 (Atalay et al. 2018, Kazimirova, et al. 2019), liver (HepG2) [Ghassemi-Barghi, et al. 2016), as in vivo in various mammalian species such as Sprague-Dawley rat, Wistar rat, [Kazimirova, et al. 2019) in various plant species such as Allium cepa L. (Heikal, et al. 2020), Glycine max (López-Moreno et al. 2010), Triticum aestivum L. (Abdelsalama, et al. 2018), Lens culinaris L. (Bellani, et al. 2020) and Oryza sativa (Waani, et al. 2021), by sister chromatid exchange, micronucleus and comet tests, as well as, γH2AX test (Lim, et al. 2012). Studies on the movement and distribution of cobalt nanoparticles from the application site to other parts of the body have shown that these nanoparticles can be found in nearby lymph nodes, spleen, and after a while in the liver, bone marrow, blood, urea and hair (Singh, et al. 2009, Rewell, 2006). In patients whose worn cobalt alloy hip prostheses were replaced, DNA double-strand breaks in fibroblast cells close to the implant, increased chromosomal abnormalities in bone marrow cells, and increased chromosomal damage in peripheral blood lymphocytes were observed (Doherty et al. 2001) Ponti et al., (2009) investigated the cytotoxicity, genotoxicity and morphological transforming activity of cobalt nanoparticles (Co-nano) and cobalt ions (Co^{2+}) in Balb/3T3 cells. They also evaluated Co-nano dissolution in culture medium and cellular uptake of both Co-nano and Co^{2+} and reported that toxicity potential of Co-nano was higher than for Co^{2+} after 2 and 24 h of exposure, while dose–effect relationships were overlapping after 72 h. It is stated that the higher uptake of co-nano by cells may be an indicator of its toxicity. It has been reported that nanoparticles use certain mechanisms such as passive diffusion, receptor-mediated endocytosis, and clathrin-coated vesicles for their entry into the cell. Colognato et al., (2008) studied to assess whether metal cobalt nanoparticles (CoNP 100–500 nm) are genotoxic compared to cobalt ions (Co^{2+}). They carried out the uptake experiments by incubating with $^{57}Co^{2+}$ or with ^{60}CoNP for 24 and 48 h on peripheral blood leukocytes. They found that intracellular Co^{2+} showed slight or no variations over the baseline levels, but CoNP were taken up efficiently leading to intracellular CoNP concentrations of 485 ± 106.1 and 970 ± 99 fg per cell after 24 and 48 h, respectively. They investigated genotoxic effects using block micronucleus test by counting

frequency of micronucleus in binucleated cells and comet assay by measuring % Tail DNA. The comet assay showed a statistically significant dose-related increase in % Tail DNA for CoNP ($p < 0.001$), whereas Co^{2+} did not induce significant changes over control values. These findings suggest that nanosized Co can be internalized by human leukocytes and can interact with DNA leading to the observed genotoxic effects, which are, however, modulated both by donor's characteristics and/or by Co^{2+} release. Papargeorgiou et al. (2007) compared the cytotoxic and genotoxic effects of NP and micron-sized particles of Co chrome alloy in cultured human fibroblasts. They observed that NP induced more DNA damage than micron-sized particles and more aneuploidy and cytotoxicity at the equivalent volumetric dose and reported that both micron and nano sized Co particles cause oxidative damage in DNA molecule, but the effect of micron sized particles is less and indicated that the increase in oxidative damage also showed parallelism with the increase in the amount of ROSs in the cell. CoOnp are known to induce the production of reactive oxygen species (ROS), which are responsible for the inhibition effects of Co NPs against different kinds of bacteria, fungi, and viruses (Turecka, et al. 2018, Anvar et al. 2019). Cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, selenium and zinc belong to the group of essential metals, but their presence in the body below or above certain concentrations can also lead to disease states (Fraga, 2005). In fact, for most metals, exposure has been linked to the induction of adverse health effects as well as to different human pathologies. From bacteria to humans, metal exposure has been associated with cell damage located in different organelles and membranes, with the proven capability of reaching the nucleus and interacting with DNA (Tchounwou, et al. 2012). From this point of view, different metal compounds are considered genotoxic and carcinogenic (IARC 2006, Auffan, et al.2009, IARC 2012).

The numerical data of comet analysis and micronucleus techniques, which were also used in our study, support each other, which shows parallelism with previous studies. In this study, the increase in micronucleus frequency and the increase in DNA damage parameters GDI and DCP values in bone marrow cells of rats treated with cobalt nanoparticles reveal a parallel data. In the correlation analysis, the inverse but strong correlation between the increase in micronucleus frequency and the number of PCE is an indication that the cobalt nanoparticle causes toxicity in the bone marrow. Lison et al., (2001) reported that two different mechanisms are responsible for the genotoxic and carcinogenic effects of cobalt compounds, namely DNA breakage induced by cobalt metal and especially hard metal particles, and inhibition of DNA repair by cobalt (II) ions. Studies on human alveolar (A549) and bronchial (BEAS-2B) cells exposed to cobalt oxide NPs have shown moderate cytotoxicity in normal bronchial cells, direct DNA damage only at high concentrations, and significant oxidative effects at low concentrations [Cavallo and Ciervo 2014). Khalil et al .(2011) reported that the high dose of cobalt doped mercaptoacetic acid (MAA)-QDs was significantly able to induce DNA damage, MN and DNA adduct (8-OHdG) generation after 2 days of oral administration in mice. This means that high doses of cobalt-doped MAA-QDs have the potential to cause indirect in vivo genetic damage.

The results obtained from the researches; shows that cobaltchrome nanoparticles cause DNA breaks, structural and numerical chromosomal abnormalities, and cause DNA damage. Nanoparticles can either directly bind to DNA or interact with DNA-bound proteins to prevent replication, transcription and translation events, bind to other cellular proteins to affect the cell division process, cause oxidative stress, abnormal signaling and abnormal response, and ultimately cause cellular damage.

CONCLUSION

The U.S. National Toxicology Program (NTP) (2016) recently released a report alluding to the carcinogenicity of cobalt compounds that release cobalt ions in vivo. In the world, most pharmaceutical companies and contract research organizations have created study designs for acute and chronic toxicology and carcinogenicity studies in rodents and non-rodents. Despite such efforts,

there is a huge gap regarding the reports and classified database of long-term toxicity of nanomaterials. These findings have important implications for understanding the potential health effects of Nano-Co exposure. Further extensive research in this field is promising and reasonable.

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