Research Paper

Nickel Increases Chromosomal Abnormalities by Interfering With the Initiation of DNA Repair Pathways

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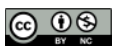

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ABSTRACT

Background: Nickel is a carcinogenic, heavy metal released through industrial activities and via natural resources. It is able to cause DNA damages by reducing the efficiency of DNA repair mechanisms. However, the exact time point at which it is able to interfere with these mechanisms is not yet clearly understood.

Methods: To find the most nickel-vulnerable time of repair mechanisms, human dermal fibroblasts (HDF) were treated with three doses of nickel before and after X-irradiation. The induced frequency of chromosomal abnormality was studied using micronucleus assay in binucleated cells. The cytotoxicity of different treatments was established using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The results revealed that nickel treatment had a synergistic effect on inducing Micronucleus frequency only when cells were treated 2 hours before X-irradiation. The X-ray treatment of the cells with 5 and 10 mM nickel had a cytotoxic effect mainly when given 6 hours after the irradiation.

Conclusion: The results suggest that nickel can interfere with human DNA repair mechanisms only at the start of the process, while having no significant effect on the human DNA repair mechanisms when activated.

Keywords: Chromosomes, DNA repair mechanisms, Human dermal fibroblast (HDF), Micronucleus assay, Nickel, X-irradiation

Introduction

Environmental pollution with heavy metals is one of the major threats to human health. Various industrial and agricultural activities increasingly pollute soil, underground water, and air. For instance, agricultural activities release nickel and lead to the environment through irrigation with wastewaters [1]. Heavy metals are absorbed through the human respiratory tract, digestive system, and skin. The potential of these heavy metals and other pollutants to induce cancer and their association with cancer progression has been widely studied on tumors in different human tissues [2-4]. Specifically, it is known that exposure to heavy metals can induce double-stranded DNA breaks. Although the exact mechanism is not fully understood yet, it is suggested that the genotoxic effect results from the heavy metals' ability to impair DNA repair mechanisms rather than damaging DNA directly [5].

Nickel is one of the most abundant heavy metals in the environment due to its popular application in various industries and important roles in the nature [6]. This heavy metal is widely used in many metallurgical processes, such as producing various alloys, electroplating, production of nickel-cadmium batteries, and as a catalyst in chemical and food industries [6]. Workers exposed to nickel are known to have higher levels of DNA damage markers than those unexposed individuals [7]. Although this metal cannot directly damage the human genetic material, it is classified as a carcinogen [8]. The carcinogenic effect of nickel is related to its ability to interfere with the repair mechanisms, resulting in DNA strands damages. In this context, nickel has the least damaging effect, compared to other heavy metals, on DNA repair proteins, such as p53 [9]. Nickel is able to down-regulate the synthesis of proteins involved in DNA repair pathways [10]. This metal also reduces the nucleotide excision and repair activities by disrupting the function and structure of p53 protein [11].

Aim of the Study: Despite the significant knowledge available on the probable effect of nickel on DNA repair mechanisms in cell, it is still not clear when it has the most disruptive effect on those processes. To address this important research question, we treated a human dermal fibroblast cell line at varying time points before and after inducing DNA damage by X-ray irradiation and analyzed the frequency of chromosomal damages for each of the experimental treatment sets versus controls.

Materials and Methods

Cell culture: The human dermal fibroblast (HDF) cell line was obtained from the cell culture lab at Ferdowsi University of Mashhad. The cells were cultured in high glucose Dulbecco's Modified Eagle Medium (Gibco; Abingdon, UK) supplemented with 10% fetal bovine serum (FBS; Gibco) at 37°C and 5% CO_2. Cell treatments were performed 48 hours after the initiation of the culture. Also, duplicate cultures were set up for each treatment set.

Treatments: Nickel-II (Merck, Darmstadt, Germany) at a dose of 2, 5, or 10 mM was added to cell cultures at different time points 2 hours before and 2, 4, or 6 hours after X-irradiation. The cells treated with each nickel dose were X-irradiated at 5 or 7 Gray (Gy), using a Philips superficial X-ray unit. Finally, the cells were harvested 30 hours post-irradiation.

Micronucleus Assay: Cell harvest for micronucleus assay was performed according to the procedure presented by Fenech (2000) with some modifications [12]. Cytochalasin-b was added to the cell culture at a final concentration of 4 µg/ml 24 hours before harvest. Cells were trypsinized and collected in centrifuge tubes and fixed on cleaned slides after hypotonic treatment and washed twice in methanol-acetic acid (5:1, v/v). Slides were stained with 10% Giemsa for 20 min. The slides were studied under 1000×magnification. In each slide, 500 binucleated cells were scored. Binucleates (Bi) harboring small, detached nuclei were considered as micro-binucleate cells (MBC). At least two slides from each flask were scored. The frequency of MBC occurrences was calculated and recorded.

Cellular metabolic activity: Approximately, 8000 cells were cultured in 96-well dishes and treated according to the protocol explained earlier when they reached 70% confluency. The cells were tested by MTT assay to measure the cellular metabolic activity. Each MTT treatment was performed in 4 separate wells. The cell viability test was performed for both the controls and treated cells at 24, 48, or 72 hours after the nickel treatment, and 72 hours after the X-irradiation procedure.

Tetrazolium salt (Sigma Aldrich, Frankfurt, Germany), dissolved in PBS, was added to the cell culture medium and left for 6 hours at 37°C. The medium in each well was then replaced with pure DMSO. The absorbance of the cell culture solution in each well was read on a spectrophotometer at 545 nm, using an ELISA reader (Awareness Inc.; Palm City, USA). Next, the absorbance for the solution in each well was compared to those of the controls at 24 hours after the culture initiation, and the data were plotted.

Statistical analyses: The statistical analyses were performed, using MINI-TAB software, v. 14. The numerical differences between the treated groups and controls, and among the treated groups themselves were also analyzed by one-way analysis of variance (ANOVA).

Results

Nickel Treatment: Cells were treated with nickel at a dose of 2, 5, or 10 mM for 24 hours. As shown in Figure 1, the nickel doses at 2 and 5 mM did not significantly increase the frequency of MBCs. However, cells treated with a nickel dose at 10 mM showed a significant increase in their MBC frequency ($P<0.05$; Figure 1).

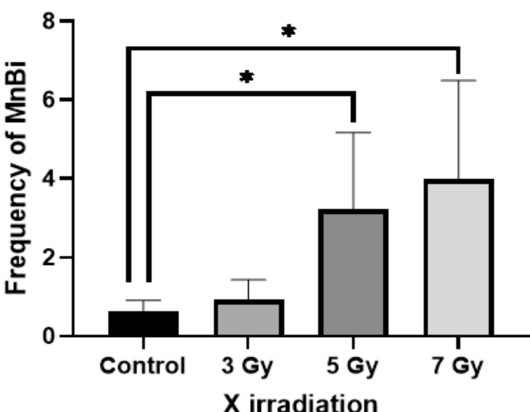

Figure 1. Frequency of MBC in cells treated with different doses of Nickel for 24 hours

* Significant statistical differences compared to the controls (P<0.05).

X-Irradiation: As shown in Figure 2, the X-irradiations at 5 and 7 Gy led to a significant increase in MBC frequency compared to the controls (P<0.05). However, the 3 Gy irradiation did not lead to a significant rise in the MBC frequency compared to those of the controls.

Irradiation of Nickel-Treated Cells: Cells were treated with different doses of Nickel 2 hours before or 2, 4, or 6 hours after 3 and 5 Gy x-irradiation. The analyzed data revealed that treating the cells 2 hours before irradiation led to a significant increase in the MBC frequency compared to those observed for the untreated but irradiated cells (Figure 3A). A significant increase in MBC frequency occurred after treatment with nickel at 10 mM (P<0.01). In addition, there was no significant increase in MBC frequency at all other nickel treatment doses compared to the corresponding untreated but X-irradiated cells (Figures 3B, 3C & 3D).

Cellular Viability Test after Nickel Treatment: The viability of cells treated with nickel at three doses of 2, 5, or 10 mM was investigated based on the MTT test. The results showed that Nickel, at the doses used, did not have a harmful effect on the cells' viability (Figure 4).

Cellular Viability Test after X-irradiation: The irradiation of the cells with two doses of 1 or 3 Gy did not affect the cells' ability to survive. However, X-irradiation of the cells at the two high doses used in this study, reduced the cell viability after 72 hours (P<0.05; Figure 5).

Cellular Viability Test after Co-treatment with Nickel and X-irradiation: The cell viability analysis at different time points, before and after X-irradiation, revealed that the two low doses of nickel protected the cells from the toxic effect of 5 Gy X-irradiation for 4h post-irradiation (Figures 6A,B&C). However, treat-

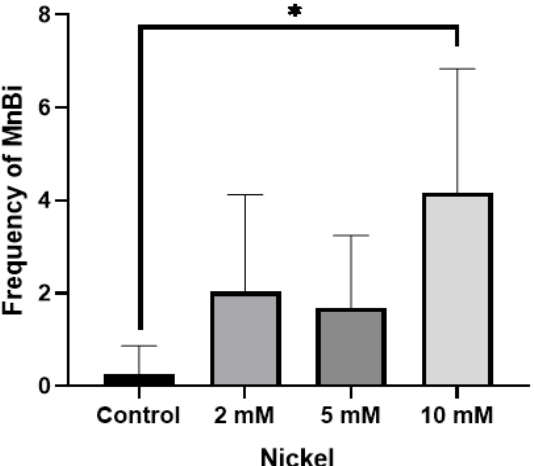

Figure 2. Frequency of MBC after X-irradiation at 3, 5, or 7 Gy

*: Significant statistical differences compared to the controls (P<0.05).

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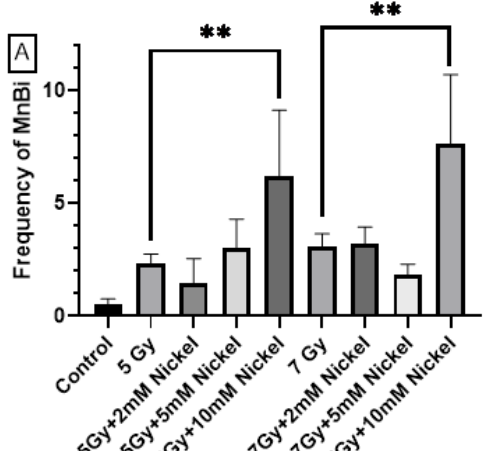
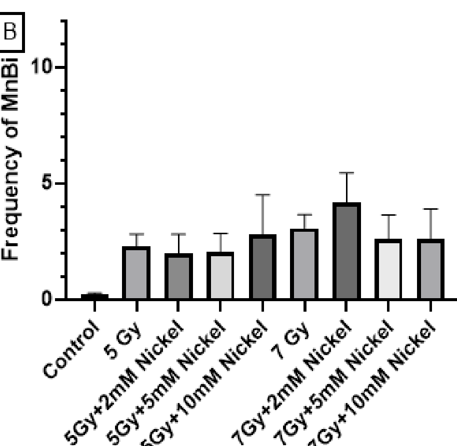
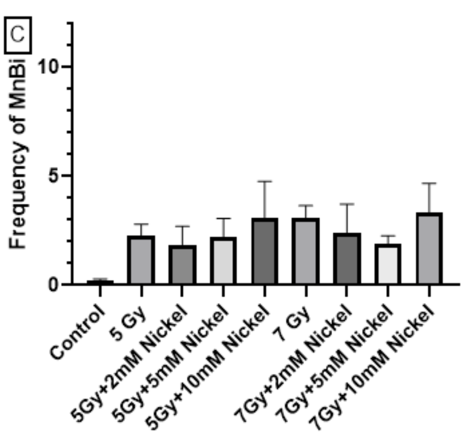






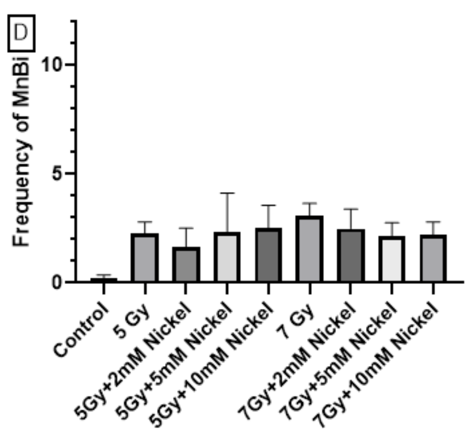


Figure 3. Nickel treatment

A) 2 hours before X-irradiation; B) 2 hours post-X-irradiation; C) 4 hours post-X-irradiation; D) 6 hours post-X-irradiation.

** Significant statistical differences with untreated but irradiated cells ($P<0.01$).

ment of the cells with nickel 6 hours post-irradiation did not have a protective effect at 5 or 7 Gy X-irradiation. The cell viability declined significantly in all treatment groups ($P<0.05$) compared to the controls except for those in the 5 Gy irradiation and 2 mM nickel groups (Figure 6D).

Discussion

Nickel, a heavy metal widely present in the environment, is suspected of having carcinogenic potentials. The ability of this metal to induce cancer is mostly related to its interference with DNA repair mechanisms, rather than causing direct damages to chromosomes through oxidative stress [13, 14]. In this context, the results of a study on treating cells from Chinese hamster with low doses of nickel provided no evidence of chromosomal mutations or abnormalities [15].

The present study demonstrated experimental evidence that nickel can induce chromosomal damages on human dermal fibroblasts at high doses (≥10 mM). However, co-treatment of the cells with nickel and X-ray irradiation led to a statistically significant increase in the frequency of MBCs, reflecting a rise in the chromosomal damages. Two hours of pre-irradiation caused a higher frequency of MBCs, but at all other nickel treatment time points post X-ray irradiation there was no increase in the MBCs more than those induced by the irradiation alone. It is likely that X-ray

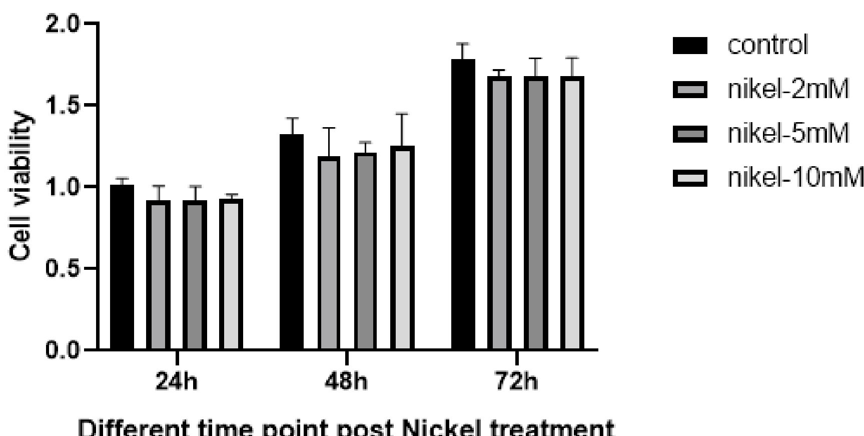


Figure 4. Cell viability after treatment with three doses of Nickel

irradiation of the cells in the absence of nickel leads to the activation of the DNA repair mechanisms. However, when nickel is present during X-irradiation, all X-ray induced-lesions in the DNA lead to chromosomal damages, which are translated into a higher frequency of MBCs compared to when no nickel is present. The results suggest that nickel interferes with DNA repair mechanisms at the start of such processes of genes transcription. In addition, after inducing repair mechanisms, nickel was not able to make any changes in the DNA repair capability of the treated cells.

Ionizing radiations, including X-rays, induce chromosomal damages by breaking DNA strands [16, 17]. The DNA double-strand break (DSB) repair capability in the cells is the main mechanism known to fix the NDA breaks. The response to the DSB repair is regulated by 53BP1 protein, which is assembled at the break points [17]. This protein is in close contact with p53 when DNA damage occurs [18].

Previous studies have shown that nickel is likely to down-regulate or interfere with the action of genes involved in repairing nucleotides break, base-pair excision, DNA mismatch, homologous-recombination, and non-homologous end-joining pathways, and the disruption in the structure and function of p53 protein [11, 13]. Also, nickel is likely to reduce cellular antioxidant defense mechanisms [19].

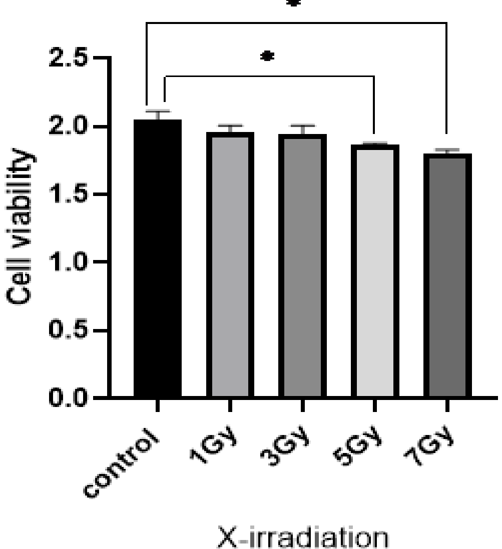
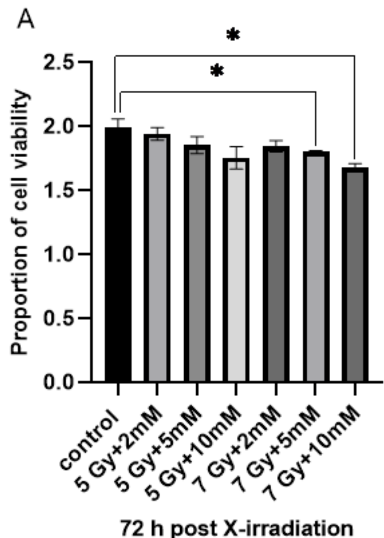
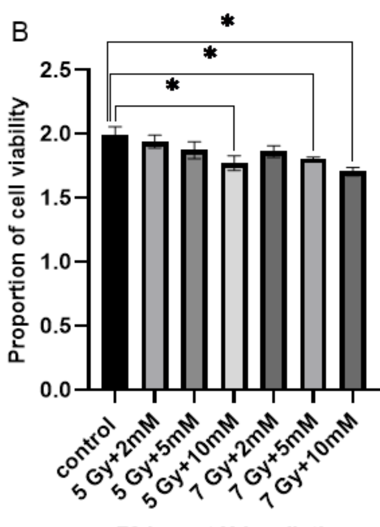
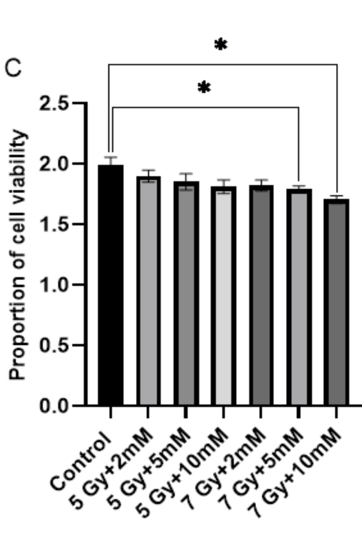


Figure 5. Cell viability, 72 hours post X-irradiation compared to control at 24 hours post X-irradiation

*: Significant statistical differences compared to the controls (P<0.05).







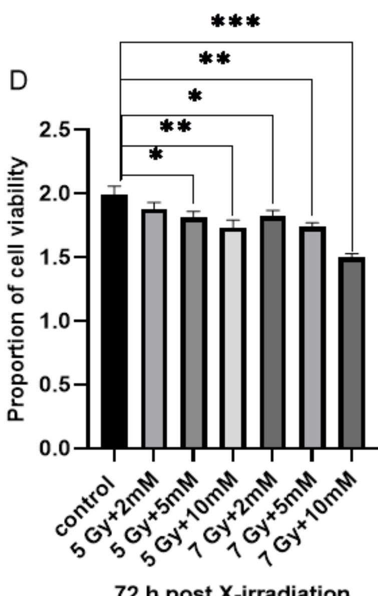


Figure 6. Cell viability after X-irradiated at 5 and 7 Gy and different doses of Nickel compared to control at 24 hours post X-irradiation

A) 2 hours pre-irradiation, B) 2 hours post-irradiation, C) 4 hours post-irradiation, and D) 6 hours post-irradiation. *: Significant statistical differences compared to the controls (P<0.05) with the following P: *P<0.05, **P<0.01, ***P<0.001.

Based on the current study results, the main reason for X-ray-induced chromosomal damages in the presence of nickel might be its ability to reduce or block gene transcriptions involved in repair mechanisms rather than interfering in the activity levels of transcriptions. Co-treatment of the cells with nickel and X-irradiation is known to have toxic effects, especially at the highest dose as used in this study. Nickel treatment also induces oxidative stress by promoting the generation of reactive oxygen species (ROS). At the same time, it can decrease the antioxidant activity of superoxide dismutase and catalase enzymes [20]. A large amount of ROS generated by ionizing radiation, leads to harmful damages to biomolecules and cell death, and impairs the antioxidant defense mechanisms of the cells.

Conclusions

Nickel can induce chromosomal damages by increasing the level of reactive oxygen species. The results of

this study show that nickel is also able to interfere with cellular DNA damage repair mechanism and provides the condition for other damaging factors, such as X-irradiation, to induce high levels of DNA lesions. Nickel is able to do so when only added prior to the activation of DNA repair systems. Lastly, nickel prevents the cellular antioxidant protective mechanisms which leads to the elevation of toxic effect of ionizing irradiation.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee, Ferdowsi University of Mashhad, Mashhad, Iran (Code: IR.IAU.MSHD.REC.1398.095).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

Authors declare no conflict of interest with any internal or external entity in conducting this research.

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References

[1] Abdelwaheb M, Jebali K, Dhaouadi H, Dridi-Dhaouadi S. Adsorption of nitrate, phosphate, nickel and lead on soils: Risk of groundwater contamination. Ecotoxicology and Environmental Safety. 2019; 179:182-87. [DOI:10.1016/j.ecoenv.2019.04.040] [PMID]

[2] Romaniuk A, Lyndin M, Sikora V, Lyndina Y, Romaniuk S, Sikora K. Heavy metals effect on breast cancer progression. Journal of Occupational Medicine and Toxicology. 2017; 12:32. [DOI:10.1186/s12995-017-0178-1] [PMID] [PMCID]

[3] Lim JT, Tan YQ, Valeri L, Lee J, Geok PP, Chia SE, et al. Association between serum heavy metals and prostate cancer risk–A multiple metal analysis. Environment International. 2019; 132:105109. [DOI:10.1016/j.envint.2019.105109] [PMID]

[4] Khlifi R, Hamza-Chaffai A. Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: A review. Toxicology and Applied Pharmacology. 2010; 248(2):71-88. [DOI:10.1016/j.taap.2010.08.003] [PMID]

[5] Morales ME, Derbes RS, Ade CM, Ortego JC, Stark J, Deininger PL, et l. Heavy metal exposure influences double strand break DNA repair outcomes. Plos One. 2016; 11(3):e0151367. [DOI:10.1371/journal.pone.0151367] [PMID] [PMCID]

[6] Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. Nickel: Human health and environmental toxicology. International Journal of Environmental Research and Public Health. 2020; 17(3):679. [DOI:10.3390/ijerph17030679] [PMID] [PMCID]

[7] Shan WU, Bai YN, Pu HQ, Jie HE, Zheng TZ, Li HY, et al. Dynamic changes in DNA damage and repair biomarkers with employment length among nickel smelting workers. Biomedical and Environmental Sciences. 2015; 28(9):679-82. [DOI:10.3967/bes2015.095][PMID]

[8] IARC working group on the evaluation of carcinogenic risks to humans. Arsenic, Metals, Fibres, and Dusts. A review of human carcinogens. Lyon: International Agency for Research on Cancer (IARC) Publications; 2012. [Link]

[9] Adámik M, Bažantová P, Navrátilová L, Polášková A, Pečinka P, Holaňová L, et al. Impact of cadmium, cobalt and nickel on sequence-specific DNA binding of p63 and p73 in vitro and in cells. Biochemical and Biophysical Research Communications. 2015; 456(1):29-34. [DOI:10.1016/j.bbrc.2014.11.027] [PMID]

[10] Scanlon SE, Scanlon CD, Hegan DC, Sulkowski PL, Glazer PM. Nickel induces transcriptional down-regulation of DNA repair pathways in tumorigenic and non-tumorigenic lung cells. Carcinogenesis. 2017; 38(6):627-37.[DOI:10.1093/carcin/bgx038] [PMID] [PMCID]

[11] Kim YJ, Lee YJ, Kim HJ, Kim HS, Kang MS, Lee SK, et al. A molecular mechanism of nickel (II): Reduction of nucleotide excision repair activity by structural and functional disruption of p53. Carcinogenesis. 2018; 39(10):1310. [DOI:10.1093/carcin/bgy118] [PMID]

[12] Fenech M. The in vitro micronucleus technique. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2000; 455(1-2):81-95. [DOI:10.1016/S0027-5107(00)00065-8]

[13] Guo H, Liu H, Wu H, Cui H, Fang J, Zuo Z, et al. Nickel carcinogenesis mechanism: DNA damage. International Journal of Molecular Sciences. 2019; 20(19):4690. [DOI:10.3390/ijms20194690] [PMID] [PMCID]

[14] Kim HL, Seo YR. Molecular and genomic approach for understanding the gene-environment interaction between Nrf2 deficiency and carcinogenic nickel-induced DNA damage. Oncology Reports. 2012; 28(6):1959-67. [DOI:10.3892/or.2012.2057] [PMID] [PMCID]

[15] Buxton S, Voges Y, Donath C, Oller A. Gene (HPRT) and chromosomal (MN) mutations of nickel metal powder in V79 Chinese hamster cells. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2020; 819-20:111688. [DOI:10.1016/j.mrfmmm.2020.111688] [PMID]

[16] Roobol SJ, van den Bent I, van Cappellen WA, Abraham TE, Paul MW, Kanaar R, et al. Comparison of high- and low-let radiation-induced DNA double-strand break processing in living cells. International Journal of Molecular Sciences. 2020; 21(18):6602. [DOI:10.3390/ijms21186602] [PMID] [PMCID]

[17] Shibata A, Jeggo PA. Roles for 53BP1 in the repair of radiation-induced DNA double strand breaks. DNA Repair. 2020; 93:102915. [DOI:10.1016/j.dnarep.2020.102915] [PMID]

[18] Wang B, Matsuoka S, Carpenter PB, Elledge SJ. 53BP1, a mediator of the DNA damage checkpoint. Science. 2002; 298(5597):1435-8. [DOI:10.1126/science.1076182] [PMID]

[19] Cavallo D, Ursini CL, Setini A, Chianese C, Piegari P, Perniconi B, et al. Evaluation of oxidative damage and inhibition of DNA repair in an in vitro study of nickel exposure. Toxicol in Vitro. 2003; 17(5-6):603-7.[DOI:10.1016/S0887-2333(03)00138-3]

[20] Terpilowska S, Siwicki AK. Pro- and antioxidant activity of chromium (III), iron (III), molybdenum (III) or nickel (II) and their mixtures. Chemico-Biological Interactions. 2019; 298:43-51. [DOI:10.1016/j.cbi.2018.10.028] [PMID]