DOI: http://dx.doi.org/10.32571/ijct.1186924 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Evaluation of the genotoxic effect of nonylphenol applied in different doses on the bone marrow

Nazmiye BITGEN[1,2*], Ozge GOKTEPE[3,2], Munevver BARAN[4], Eren Kaan IPEK[1], Arzu YAY[3,2]

[1]*Department of Medical Biology, Faculty of Medicine, Erciyes University, Kayseri, Türkiye*

[2]*Genome and Stem Cell Center (GENKOK), Erciyes University, Kayseri, Türkiye*

[3]*Department of Histology and Embryology, Faculty of Medicine, Erciyes University, 38039 Kayseri, Türkiye*

[4]*Department of Pharmaceutical Basic Science, Faculty of Pharmacy, Erciyes University, Kayseri, Türkiye*

Received: 10 September 2022; Revised: 24 April 2023; Accepted: 9 May 2023

*Corresponding author e-mail: nazmiyebitgen@gmail.com.tr

Citation: Bitgen, N.; Goktepe, Ö.; Baran, M.; Ipek, E. K.; Yay, A. *Int. J. Chem. Technol.* **2023**, 7 (1), 108-112.

ABSTRACT

Nonylphenols are endocrine disrupting toxic compounds and are widely used. For this reason, our study aimed to investigate the genotoxic effect of nonylphenols applied at different doses on mouse bone marrow cells. Groups were classified as control group, the sham administered only group, low dose, medium dose and high dose nonylphenol administered group. After euthanasia, polychromatic erythrocyte/Normochromatic erythrocyte ratio and micronuclear polychromatic erythrocyte count were determined in femur bone marrow cells and analyzed to detect DNA damage. While polychromatic erythrocyte/Normochromatic erythrocyte ratio was found to be lower in the groups treated with different doses of nonylphenol, especially in the group treated with high-dose nonylphenol, this decrease was not statistically significant. When the micronucleated polychromatic erythrocyte values were examined, it was observed that there was a statistically significant increase, especially in the medium and high dose groups compared to the control. High-dose nonylphenol has a toxic effect and may have a long-term genotoxic effect such as cancer development by interacting with genotoxic agents.

Keywords: Nonylphenol, genotoxicity, micronucleus.

Farklı dozajlarda uygulanan nonylphenol'ün kemik iliği üzerine genotoksik etkisinin değerlendirilmesi

ÖZ

Nonilfenoller; endokrin bozucu toksik bileşiklerden olup yaygın olarak kullanılmaktadır. Bu sebeple çalışmamızda farklı dozlarda uygulanan Nonilfenoller'in, fare kemik iliği hücreleri üzerine genotoksik etkisinin araştırılması amaçlanmıştır. Gruplar; kontrol grubu, sadece sham uygulanan grup, düşük doz, orta doz ve yüksek doz nonilfenol uygulanan grup olacak şekilde sınıflandırıldı. Ötenazi sonrası, femur kemik iliği hücrelerinde Polikromatik eritrosit/Normokromatik eritrosit oranı ile mikroçekirdekli polikromatik eritrosit sayısı belirlenerek DNA hasarını tespit etmek için analizleri yapıldı. Farklı dozlarda nonilfenol uygulanan gruplardan özellikle yüksek doz nonilfenol uygulanan grupta Polikromatik eritrosit/Normokromatik eritrosit oranı kontrole düşük bulunurken, bu azalma istatistiksel olarak anlamlı değildi. Mikroçekirdekli polikromatik eritrosit değerlerine bakıldığında ise özellikle orta ve yüksek doz gruplarında kontrole göre istatistiksel olarak anlamlı derecede bir artış olduğu gözlendi. Yüksek doz nonilfenol toksik etki göstermekte olup, genotoksik ajanlarla etkileşerek kanser gelişimi gibi uzun vadeli genotoksik etkiye sahip olabilir.

Anahtar Kelimeler: Nonilfenol, genotoksisite, mikronükleus.

1. INTRODUCTION

Short-term, low-dose exposure to xenobiotics may not cause any permanent effects on DNA, but chronic exposure to xenobiotics can cause mutations and future cancer.[1] Nonylphenol (NP) is one of the xenobiotic compounds frequently encountered in many environments directly or indirectly.[2] NP is a degradation product of plastic compounds used in the manufacture of dentistry, food packaging, textiles, pesticides, detergents,

DOI: http://dx.doi.org/10.32571/ijct.1186924 E-ISSN: 2602-277X

paints, and cosmetics.[3] NP is of great interest as a hazardous pollutant due to its long-term persistence in the environment and multiple toxic effects.[4] Trace consumption of NPs in food and water is thought to be the main source of human exposure.[5] It has been stated that NP has a DNA damaging effect due to its biotransformation to reactive intermediates that can cause changes in DNA.[6] Most of the previous studies have focused on the toxicity of 4-NP and its effects on the developmental process and reproductive system, especially in aquatic ecosystems, were evaluated.[7,8] Little information is available about its genotoxic effect on bone marrow. The micronucleus (MN) test, which is one of the popular tests preferred in the evaluation of environmental genotoxicity, is an indicator of cytogenetic damage.[9] MN is produced from fragments of chromosomes or whole chromosomes that are delayed in cell division due to centromere deficiency, damage to the centromere, or defects in cytokinesis. In actively dividing cells, the MN number reflects the effect of clastogenic or aneugenic compounds.[10] The MN test, which is easy to apply, is used to determine the damage of these agents. An increase in the number of MN in cells is a marker of genomic instability in somatic cells.[9]

When hematopoietic cells divide, the application of various chemicals results in chromosome damage or the suppression of mitosis.[11] Bone marrow (BM) is the main hematopoietic organ in adult rodents. Erythrocytes are used in the micronucleus test, which is studied with mouse bone marrow. After transforming into polychromatic erythrocytes (PCE), erythroblasts lose their nuclei approximately 6 hours after mitosis. In this way, it is easier to determine the formation of MN.[12]An increase in the amount of immature PCE containing MN (MNPCE) is a sign of chromosomal or cytogenetic damage caused by anaphase delay.[11] The significant decrease observed in the polychromatic erythrocyte/normochromatic erythrocyte (PCE/NCE) ratio indicates that the applied chemical exerts a cytotoxic effect on the division and maturation of nucleated cells.[13] This study aimed to investigate the genotoxic effect of nonylphenol applied at different doses on rat BM cells.

2. MATERIALS AND METHODS

An ethics committee approval (20/077) was obtained from the Erciyes University Animal Experiments Local Ethics Committee for the realization of this study. In the study, 50 male Wistar-albino rats, 8-10 weeks old, were reared in the Erciyes University Experimental and Clinical Research Center. The rats weighed between 200 and 250 g. The rats were housed in plastic cages and fed with normal pellets under standard laboratory conditions throughout the study.

2.1. Chemicals and working groups

In the study, Nonylphenol (cat no:84852-15-3, Sigma-Aldrich) were obtained from the project and used. Groups were created as following; 50 Wistar-albino adult male rats were divided into 5 groups with 10 rats in each group. Doses were determined for all applications and administered at the same time of the day. Anesthesia was administered intraperitoneally 24 hours after the last administration with ketamine hydrochloride (50 mg kg^{-1}) and 2% xylazine hydrochloride (10 mg kg^{-1}). After removing the femoral bone of each rat, the BM was placed in an eppendorf tube.

Control group: No intervention was administered.

Sham group: 150 µL of corn oil was given via gavage for 15 days.

Low Dose Nonylphenol group: 25 µL dose of nonylphenol dissolved in 125 µL corn oil was given for 15 days via gavage.

Medium Dose Nonylphenol group: 50 µL dose of nonylphenol dissolved in 100 µL corn oil was given for 15 days via gavage.

High Dose Nonylphenol group: 75 µL dose of nonylphenol dissolved in 75 µL of corn oil was given via gavage for 15 days.

2.2. Micronucleus test

Rats' femurs were removed by cervical dislocation. The supernatant was discarded by centrifugation at 2000 rpm for 7 minutes. The remainder was suspended in 0.5 mL fetal calf serum and spread on slides. After fixing with methanol, it was painted with 20% Giemsa dye for 30 minutes and left to dry at room temperature. Each rat was coded from the BM to prepare four preparations, and the prepared preparations were covered with entellan. For genotoxic activity, 1000 PCEs were randomly counted from the preparation prepared for each rat at X100 magnification in all groups under the microscope (Olympus BX51, Tokyo, Japan). Their percentages were calculated by determining the MNPCE numbers in them. In addition, the PCE/NCE ratio was determined by counting 2000 erythrocytes to show cytotoxicity.[14]

2.3. Statistical analysis

Statistical analyses of the data were performed using the Graphpad PRISM (Graphpad Software Inc., Version 8.0d) software program. The conformity of the data to the normal distribution was determined by the Shapiro-Wilk and Kolmogorov-Smirnov tests. Percent vitality values according to doses were compared with one sample t-test. A one-way Anova test was used to compare normally distributed data in multiple comparisons, and Kruskal-

DOI: http://dx.doi.org/10.32571/ijct.1186924 E-ISSN: 2602-277X

Wallis tests were used to compare non-normally distributed data. Post-hoc comparisons of the variables that were significant as a result of group comparisons were made with the Bonferroni test for the one-way Anova test and Dunn's test for the Kruskal-Wallis test. $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Micronucleus frequency in control and experimental groups

For the MN test, a total of 1.000 PCEs were counted in the BM preparations of each animal, and the percentage of PCEs with MN in them was determined (MNPCE) (Figure 1). In total, 10.000 cells were counted per group (10 rats per group). The change in the frequency of MN depending on the dose is given in Figure 2. In addition, PCE/NCE ratios were calculated by counting 2.000 erythrocytes from each rat sample, that is, 20.000 erythrocytes (PCE and NCE) for each group. Table 1 displays the MNPCE and PCE/NCE ratios determined for the control and experimental groups.

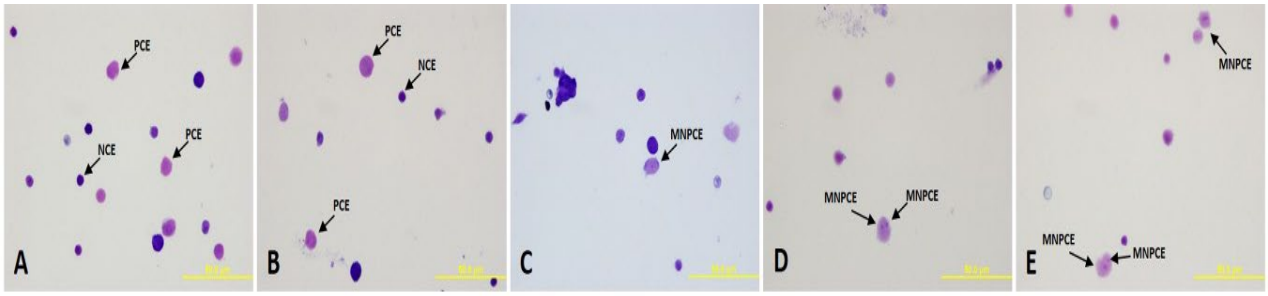

Figure 1. Polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), and micronucleated polychromatic erythrocytes (MNPCE) in rat BM (Giemsa staining X40, Olympus BX51). A) Control group, B) Sham group, C) Low-dose group, D) Medium-dose group, and E) High-dose group.

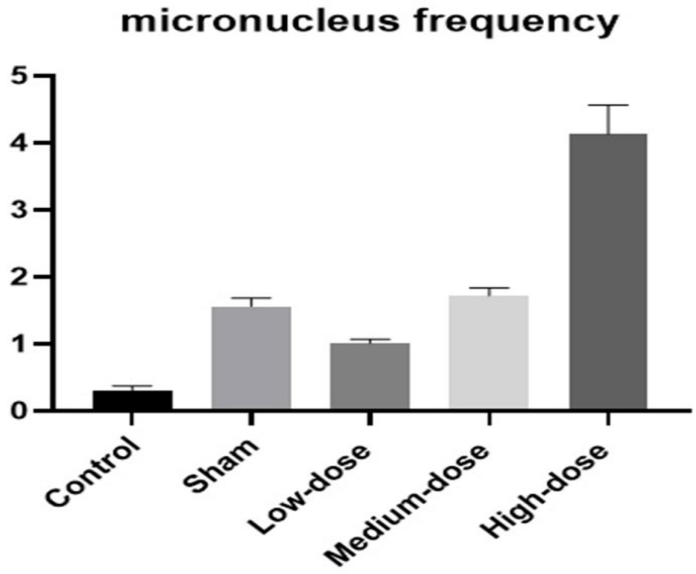

Figure 2. Change of micronucleus frequency according to groups.

Table 1. PCE/NCE ratio and MNPCE frequencies between different groups.

Groups (n=10)	Control group (mean±SD)	Sham group (mean±SD)	Low-dose group (mean±SD)	Medium-dose group (mean±SD)	High-dose group (mean±SD)	p
PCE/NCE	$(0.99 \pm 0.37)^{ac}$	$(1.44 \pm 0.34)^{ab}$	$(1.61 \pm 0.26)^{b}$	$(1.92 \pm 0.51)^{b}$	$(0.65 \pm 0.32)^{c}$	< 0.0001
MNPCE, %	$(0.31 \pm 0.20)^{a}$	$(1.56 \pm 0.38)^{b}$	$(1.01 \pm 0.19)^{ab}$	$(1.72 \pm 0.36)^{b}$	$(4.13 \pm 1.38)^{c}$	< 0,0001

DOI: http://dx.doi.org/10.32571/ijct.1186924 E-ISSN: 2602-277X

3.2. PCE/NCE ratios in control and experimental groups

When the PCE/NCE ratios were considered, it was found that the high-dose NP group was lower than the control group. However, this change was not statistically significant ($p = 0.4595$). When the MNPCE values were examined, a statistically significant increase was observed in the medium and high dose groups compared to the control (respectively $p = 0.0002$, $p < 0.0001$). At the same time, a statistically significant increase was observed when the high-dose NP-administered group was compared with the low-and medium-dose NP-administered groups ($p < 0.0001$).

Genotoxic agents are substances that disrupt genetic material by causing adverse effects on cell DNA. Genotoxic effects cause mutation. Mutations can manifest themselves with various idiosyncratic and allergic reactions, as well as with carcinogenic effects. For this reason, it is of great importance to investigate and reveal mutating agents in terms of health. Some pharmaceutical and chemical substances, cytotoxic agents used in cancer treatment, radioactive drugs, or wastes containing such substances can cause genotoxic effects. While such genotoxic substances can be directly effective, some of them are activated by metabolic reactions and cause genotoxicity.[15]

Identifying mutagenic and toxic substances is particularly important because of their potential to cause cancer and cause adverse changes in future generations. From this point of view, the risk associated with the DNA-damaging effect caused by NP needs scientific studies to deepen its knowledge. In a previous study, mice were subchronically exposed to X-rays (0.05 Gy and 0.10 Gy), nonylphenol (NP) (25 mg kg^{-1} and 50 mg kg^{-1}), or a combination of both. As a result of the study, it was stated that NP increased the DNA damage both when used alone and in combination with X-rays.[5] Dobrzynska[5] investigated the effects of NP and ionizing radiation on DNA damage in mouse (male and female) somatic cells, separately or in combination, using the Comet Assay Method. The induction of DNA damage by NP differs according to tissue and gender. Although NP alone is not mutagenic in female mice, it has been shown to increase DNA damage in some organs with combined administration, whereas in male mice the damage has been shown to be reduced after exposure. At the same time, there are different studies in which the frequency of MN increases after exposure to 4-NP.[16,17] In this study, unlike other studies, the effect of NP on DNA damage in the BM was evaluated depending on the dose by administering three different low, medium, and high doses of NP.

4. CONCLUSIONS

According to our results, the increases in NP cause significant increases in MNPCE in cells. Due to the activation of the chemical in the bone marrow, the erythrocytes in the division phase could not perform the correct division. As the nonylphenol dose increases, genomic instability increases, preventing cells from adopting the correct structures. Therefore, the genomic instability occurring in erythrocytes, and mature erythrocyte formation cannot be observed. The low number of NCEs in the microscopic field is important proof that the transition of cells to the mature erythrocyte is less. Additionally, it is also conceivable that NPs have negative effects on specific transcription control mechanisms that are affected during the mitosis stage. As a result of our findings, the toxicity of NP increases depending on the dose increases, especially high-dose NP. From this point of view, it shows that when NP interacts with other genotoxic agents, it may cause an increase in long-term genotoxic effects such as cancer development, and it shows that care should be taken about the use of NP in daily life. We believe that these findings will shed light on further studies to find new agents to be used against the genotoxicity of NP.

ACKNOWLEDGEMENTS

This study (project no: TCD-2021-11459) was supported by Erciyes University Scientific Research Projects Coordination Unit.

Ethics Committee Approval

Erciyes University Ethics Committee (2020/077).

Conflict of interests

I declares that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. Ozdemir, F.; Kayaaltı, Z.; Dilek, K. A. *Marmara Pharm. J.* **2015**, 19(3), 246-251.

2. Zemheri, F.; Cevdet, U. *Marmara Üniv. Fen Bilim. Derg.* **2018**, 30(1), 71-76.

3. Urriola-Muñoz, P.; Li, X.; Maretzky, T.; McIlwain, D.R.; Mak, T.W.; Reyes, J.G.; Blobel, C.P.; Moreno, D. *J. Cell. Physiol.* **2018**, 233(3), 2247-2256.

4. Abou Khalil, N. S.; Abd-Elkareem, M.; Sayed, A. H. *Aquac. Nutr.* **2017**, 23(6), 1467-1474.

5. Dobrzyńska, M. M. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2014**, 772, 14-19.

6. Vazquez-Duhalt, R.; Marquez-Rocha, F.; Ponce, E.; Licea, A.F.; Vaiana, M.T. *Appl. Ecol. Environ. Res.* **2005**, 4:1-25.

7. Wu, F.; Lin, L.; Qiu, J.W.; Chen, H.; Weng, S.; Luan, T. *Aquat. Toxicol.* **2014**, 155, 43-51.

DOI: http://dx.doi.org/10.32571/ijct.1186924 **E-ISSN: 2602-277X**

8. Shirdel, I.; Mohammad, R.K. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2016**, 183-184, 28-35.

9. Fenech, M.; Chang, W. P.; Kirsch-Volders, M.; Holland, N.; Bonassi, S.; Zeiger, E. *Mut. Res.* **2003**, 534, 65-75.

10. Baršienė, J.; Dedonytė, V.; Rybakovas, A.; Andreikėnaitė, L.; Andersen, O. K. *Aquat. Toxicol.* **2006**, 78, 99-104.

11. Lambert, I.B.; Singer, T.M.; Boucher, S.E.; Douglas, G.R. *Mutat. Res.* **2005**, 590, 1-280.

12. Mavourni, K.H.; Blakey, D.H.; Cimino, M.C.; Salamone, M.F.; Heddle, J.A. *Mutat. Res.* **1990**, 239, 29-80.

13. Celik, A.; Mazmanci, B.; Camlica, Y.; Askin, A.; Comelekoglu, U. *Mutat. Res.* **2003**, 539, 91-97.

14. Bara, M.; Bitgen, N.; Kalkan, K.T.; Goktepe, O.; Varol, S.; Yay, A.H. *Dicle Tip Derg.* **2022**, 49(1), 36-44.

15. Aksu, P.; Doğan, A.; Gül, S.; Kanıcı, A. *Kafkas Univ. Vet. Fak. Derg.* **2013**, 19(6), 955-961.

16. Mekkawy, I. A.; Mahmoud, U. M.; Sayed, A.H. *Tissue and Cell.* **2011**, 43, 223-229.

17. Sharma, M.; Chadha, P. *Drug Chem. Toxicol.* **2017**, 40(3), 320-325.