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Cytotoxicity and genotoxicity assessment of *Nicotiana tabacum* extracts from conventional cigarettes and heated tobacco products

Original Article

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Abstract:

Tobacco leaves (*Nicotiana tabacum*, fam. Solanaceae) have been used for thousands of years in various forms and nowadays are mainly consumed as conventional cigarettes and heated tobacco products. The aim of this study was to compare the cytotoxic and genotoxic properties of tobacco extracts obtained from conventional cigarettes (CntE) and heated tobacco products (HntE). *In vitro* assessment revealed that the cytotoxicity of CntE was significantly higher than that of HntE. *In vivo* assessment showed that the genotoxicity of HntE was significantly lower than that of CntE ($p < 0.001$). Detailed cytogenetic analysis confirmed the higher genotoxic properties of CntE. However, due to the observed high frequency of certain chromosomal aberrations following treatment with HntE, despite the lower overall cytotoxicity and genotoxicity of this extract, heated tobacco products do not demonstrate a clear safety advantage over conventional cigarettes and warrant further toxicological investigation.

Key words:
A. cepa test, cytotoxicity, genotoxicity, haemolysis, tobacco

Apstrakt:

Procena citotoksičnosti i genotoksičnosti ekstrakata vrste *Nicotiana tabacum* iz klasičnih cigareta i proizvoda sa zagrevanim duvanom

Listovi duvana (*Nicotiana tabacum*, fam. Solanaceae) hiljadama godina se upotrebljavaju u različitim oblicima, a danas ponajviše u obliku klasičnih cigareta i proizvoda sa zagrevanim duvanom. Cilj ovog istraživanja bio je da se uporede citotoksična i genotoksična svojstva ekstrakata duvana dobijenih iz klasičnih cigareta (CntE) i proizvoda sa zagrevanim duvanom (HntE). *In vitro* procena pokazala je da je citotoksičnost CntE značajno viša u odnosu na HntE. *In vivo* procena pokazala je da je genotoksičnost HntE značajno niža u odnosu na CntE ($p < 0,001$). Detaljna citogenetička analiza potvrdila je izraženiji genotoksični potencijal CntE. Ipak, iako su ukupna citotoksičnost i genotoksičnost ovog ekstrakta niže, proizvodi sa zagrevanim duvanom ne pokazuju jasnu bezbednosnu prednost zbog uočene visoke učestalosti pojedinih hromozomskih aberacija nakon tretmana HntE, što zahteva dalja toksikološka ispitivanja.

Ključne reči:
A. cepa test, citotoksičnost, duvan, genotoksičnost, hemoliza

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Introduction

Tobacco leaves (*Nicotiana tabacum*, fam. Solanaceae) have been used for thousands of years. First, tobacco was used as a sacred ritual element and a medical panacea. Afterwards, following Christopher Columbus's arrival in the Americas, dried leaves and seeds were brought to Europe, where the plant was popularised for its therapeutic application in general bodily ills, catarrh, colds, fevers, as an aid to digestion and in the prevention of hunger and thirst, as a purgative and as a narcotic (Duke et al., 2022; Charlton, 2004). Additionally, throughout past centuries, tobacco has been used in traditional practices for teeth whitening (Charlton, 1992) and even today, such practices persist in some countries, both for teeth whitening and for the relief of dental pain (Mehra et al., 2020). Its rich phytochemical composition is well documented in the literature and includes alkaloids such as nicotine; phenolic compounds such as rutin, apigenin, and quercetin; phenolic acids such as chlorogenic, ferulic, and caffeic acids; organic acids such as citric and malic acids; terpenoids such as solanesol and cembranoid diterpenes; essential oils such as α- and β-ionone; vitamins E and K_1;

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scopolamine, mesmine, and feruloyltyramine; as well as polysaccharides, polypeptides, and sterols (Zhang et al., 2024). Therefore, the widespread use of this species is not surprising.

Nowadays, tobacco leaves are predominantly consumed in the form of conventional manufactured cigarettes and heated tobacco products, the former involving the combustion of tobacco leaves and the latter heating tobacco without directly burning it (Jankowski et al., 2019).

Despite extensive tobacco-control and smoking-cessation campaigns, according to the World Health Organization, approximately 1.25 billion adults worldwide continue to use tobacco. At the same time, tobacco use is responsible for more than 7 million deaths annually worldwide, together with approximately 1.3 million deaths among non-smokers exposed to second-hand smoke (WHO, 2025). Furthermore, the adverse effects of tobacco use are not limited to smokers and individuals exposed to second-hand smoke. Namely, workers involved in tobacco cultivation may also be exposed to occupational health hazards associated with tobacco production, where dermal absorption of a complex mixture of chemicals present in tobacco leaves, together with organic and inorganic pesticides used to treat tobacco, often leads to the development of green tobacco sickness (Silva et al., 2013).

Although numerous studies have investigated the adverse effects of smoking on overall health, relatively few have directly compared the toxicity of *N. tabacum* extracts obtained from conventional and heated tobacco products.

Thus, the aim of this study was to compare the cytotoxic and genotoxic properties of *N. tabacum* extracts obtained from commercially available conventional and heated tobacco products marketed in Europe.

Materials and Methods

Extract preparation

Conventional and heated tobacco products labelled as containing 0.8 mg nicotine per cigarette were obtained from a local supermarket in Niš, Serbia. *N. tabacum* plant material was separated from the remaining cigarette components. A 10 g sample of plant material was boiled in 100 ml of distilled water until the volume was reduced by half. The resulting extracts were filtered, and the solvent was removed under reduced pressure using a vacuum rotary evaporator (IKA RV10 Rotary Evaporator with HB10 Heating Bath, Gaithersburg, USA). The concentrated extracts from conventional cigarettes (CntE) and heated tobacco products (HntE) were stored at 4 °C in the dark until further use.

Determination of total phenolic and flavonoid content

The total phenolic content (TPC) of the extracts was determined spectrophotometrically (UV-1650PC, Shimadzu 1650, Europe) as previously described by Madić et al. (2021). Briefly, TPC was calculated from a gallic acid calibration curve (10–100 mg/l). Data were expressed as gallic acid equivalents (GAE)/g of the extract. Total flavonoid content (TFC) was calculated from a quercetin hydrate calibration curve (10–100 mg/l) and expressed as quercetin equivalents (QuE)/g of dry extract.

DPPH radical scavenging test

The antioxidant activity of the tested extracts was assessed spectrophotometrically (UV-1650PC, Shimadzu 1650, Europe) using the DPPH radical scavenging method as previously described by Madić et al. (2021). The positive control was a methanolic DPPH radical solution. Butylated hydroxytoluene (BHT) was used as a standard. Seven concentrations of CntE, HntE and BHT were assessed (0.05, 0.10, 0.15, 0.25, 0.50, 0.75, and 1 mg/ml). The radical scavenging activity (DPPH r.s.a.) was calculated using the formula:

$$\text{DPPH r.s.a.}\left(\%\right) = \frac{\text{Absorbance of the positive control}}{\text{Absorbance of the tested extract}} \times 100$$

Red blood cell haemolysis assay

Animal use complied with the principles of the Care and Use of Laboratory Animals and was approved by the Ethical Committee of the Faculty of Medicine, University of Niš (No. 323-07-05754/2023-05).

The haemolytic assay was performed using a slightly modified protocol of Madić et al. (2026). Briefly, 0.5 ml of a red blood cell (RBC) suspension was mixed with 0.5 ml of each concentration of the tested extracts (0.25, 0.5, 0.75, 1.25, 2.5 mg/ml) and the mixture was incubated at 37 °C for 30 minutes and for 60 minutes. The samples were subsequently centrifuged at 2000 rpm for 5 minutes at 4 °C. 0.2 ml of the obtained supernatant was mixed with 2.8 ml of Drabkin's reagent, incubated for 10 minutes at 21 °C, and absorbance was measured spectrophotometrically at 540 nm. The negative control (NC) was RBCs in phosphate-buffered saline (PBS), and the positive control (PC) was RBCs in dH_2O. Haemolytic activity (H.A.) was calculated as follows:

$$\text{H.A.}\left(\%\right) = \frac{\text{absorbance of the tested extract} - \text{absorbance of the NC}}{\text{absorbance of the PC} - \text{absorbance of the NC}} \times 100$$

Allium cepa test

The *A. cepa* test was performed as previously described by Madić et al. (2026). Briefly, three

concentrations of the tested extracts were analysed (0.25, 0.75, 2.5 mg/ml). The negative controls were bulbs grown in distilled water and the positive controls were bulbs exposed to 6% H_2O_2. Meristematic cells were analysed using a Leica microscope (Leica DM2500, Germany) at 400× and 1000×. For each tested concentration, a total of 2500 cells were analysed.

Cytotoxicity was evaluated based on the mitotic index (M.I.), whereas genotoxicity was assessed by determining the total percentage of cells with chromosomal aberrations (Chr.Ab.) and the frequencies of specific aberrations, including C-mitosis, chromosomal breaks/fragments, chromosome stickiness, chromosomal bridges, and different types of multipolar mitosis (**Fig. 1**).

M.I. was calculated as the percentage ratio between the number of mitotic cells and the total number of observed cells, and Chr.Ab. as the percentage ratio between the number of aberrant mitotic cells and the number of observed cells. The frequency of specific chromosomal aberrations was determined as the percentage ratio between the number of cells with specific aberrations and the total number of aberrant cells (Madić et al., 2026).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, La Jolla, California, USA). *In vitro* experiments (determination of TPC and TFC, assessment of DPPH radical scavenging activity and RBC haemolysis assay) were performed in triplicate, and the *in vivo* experiment (*A. cepa* test) was performed in five replicates, and data were expressed as mean ± standard deviation. The homogeneity of variances among the control and treatment groups exposed to different concentrations of the tested extracts was assessed using the Brown–Forsythe test. Differences among the groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons test. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The total phenolic and flavonoid contents of *N. tabacum* extracts obtained from conventional and heated tobacco products were similar. The TPC of both extracts was 40.1 mg GAE/g, while the TFC values were 21.27 ± 0.25 mg QuE/g and 23.8 ± 4.5 mg QuE/g for CntE and HntE, respectively. The extraction yields were 8.27% and 8.15% for CntE and HntE, respectively. Interestingly, the levels of both TPC and TFC were much higher than those reported by Leal et al. (2023), due to the different methods of extraction used.

In the DPPH assay, the radical-scavenging activity of both tested extracts and BHT increased in a concentration-dependent manner. Interestingly, at some tested concentrations, HntE exhibited higher radical-scavenging activity than CntE, as shown in **Fig. 2.** However, based on the estimated IC_{50} values, which were 1.42 mg/ml for CntE, 1.43 mg/ml for HntE, and 1.23 mg/ml for BHT, it can be observed that both extracts had high antioxidant activity, only slightly lower than the commercially used antioxidant BHT. This finding is consistent with the work of Ru et al. (2012), who reported that flavonoids isolated from *N. tabacum* leaves exhibited antioxidant activity comparable to that of ascorbic acid.

As shown in **Fig. 3**, the results of the RBC haemolysis assay demonstrated that both tested extracts exhibited concentration-dependent haemolytic activity. Interestingly, HntE displayed lower cytotoxicity than CntE, even after extending the incubation period from 30 to 60 minutes to allow sublethal erythrocyte membrane damage to fully manifest. According to the established classification, plant extract cytotoxicity is considered weak below 20%, potent above 20%, and high when exceeding 50% (Khalili et al., 2014; Madić et al., 2019). Based on these thresholds, and the fact that even the highest tested concentrations of CntE and HntE resulted in only 12.58 ± 0.007% and 11.04 ± 0.004% haemolysis, respectively, the results indicate that all tested concentrations exhibited weak cytotoxic properties. Interestingly, the lower tested doses of HntE (0.25 and 0.5 mg/ml) even displayed antihaemolytic activity, i.e., cytoprotective properties (Madić et al., 2019) during the shorter incubation period (**Fig. 3a**).

Consistent with the results of the *in vitro* haemolysis assay using RBCs, the *in vivo* experiment using the *A. cepa* assay showed that both tested extracts exerted mild, dose-dependent cytotoxic effects compared with the negative control. At the same time, as shown in **Fig. 4a**, even the highest tested concentrations of CntE and HntE did not induce a decrease in the mitotic index as pronounced as that observed in the positive control ($p < 0.001$).

In contrast to the cytotoxicity findings, the *in vivo* genotoxicity analysis revealed a pronounced genotoxic effect of the *N. tabacum* extract isolated from conventional cigarettes. Namely, at all tested concentrations, the percentage of total chromosomal aberrations in the meristematic cells of *A. cepa* induced by CntE treatment was significantly higher than that induced by HntE ($p < 0.001$), as shown in **Fig. 4b**. At the same time, the lowest tested concentration of HntE (0.25 mg/ml) did not induce a statistically significant increase in chromosomal aberrations compared with the negative controls,

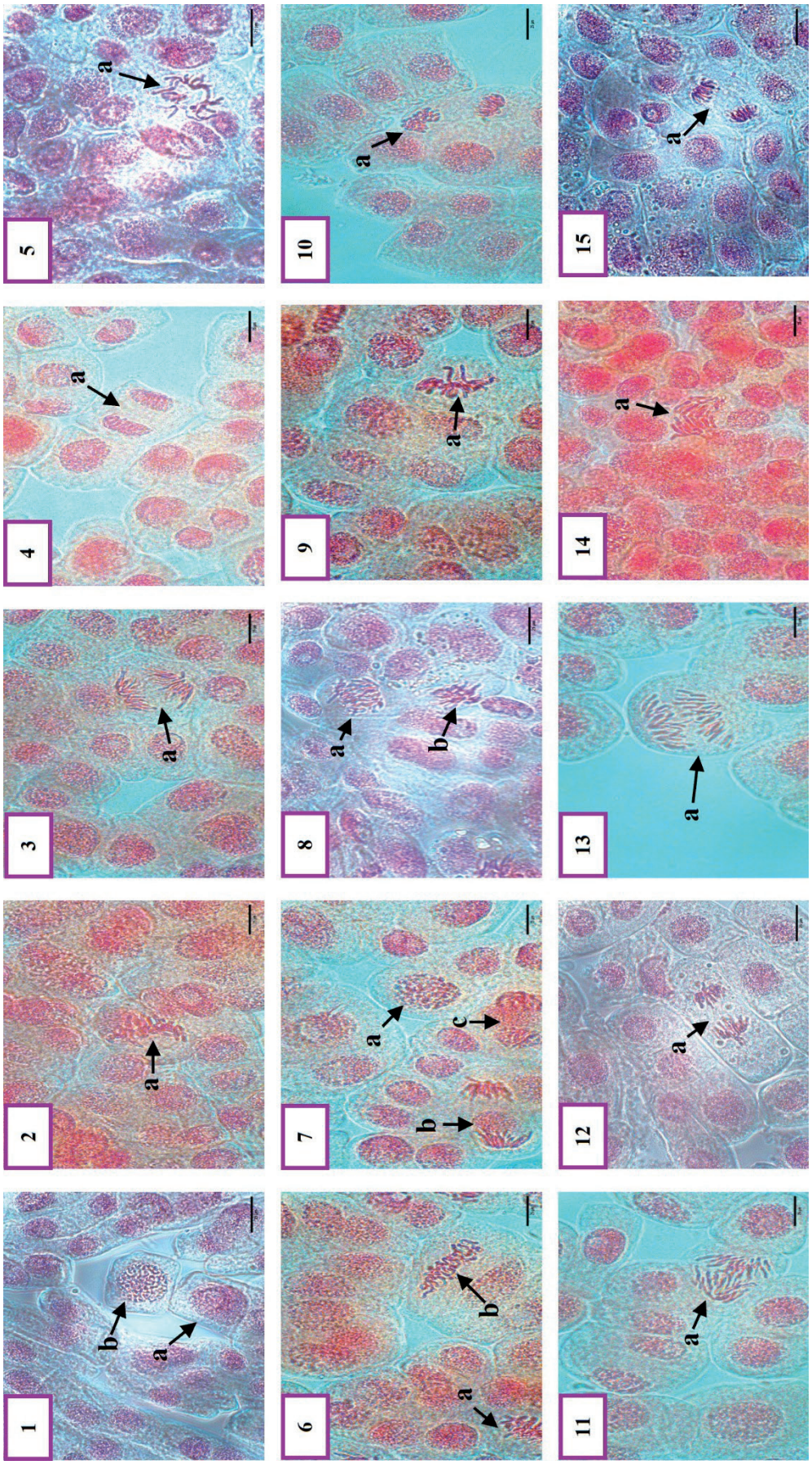


Fig. 1. Mitotic stages and specific chromosomal aberrations following treatments. **1(a)**: interphase; **1(b)**: normal prophase; **2(a)**: normal metaphase; **3(a)**: normal anaphase; **4(a)**: normal telophase; **5(a)**: C-mitosis; **6(a)**: normal metaphase; **6(b)**: fragmented metaphase; **7(a)**: fragmented prophase; **7(b)**: lagging chromosome in anaphase; **7(c)**: fragmented early telophase; **8(a)**: lagging chromosome in early anaphase; **8(b)**: sticky metaphase; **9(a)**: sticky and lagging chromosomes in metaphase; **10(a)**: fragmented telophase; **11(a)**: fragmented chromosomes and multiple bridges in anaphase; **12(a)**: vagrant chromosomes in telophase; **13(a)**: bridge in anaphase; **14(a)**: fragmented chromosomes and bridges in early anaphase; **15(a)**: fragmented late telophase. Scale bar: 25 µm. Magnification: 1000×.

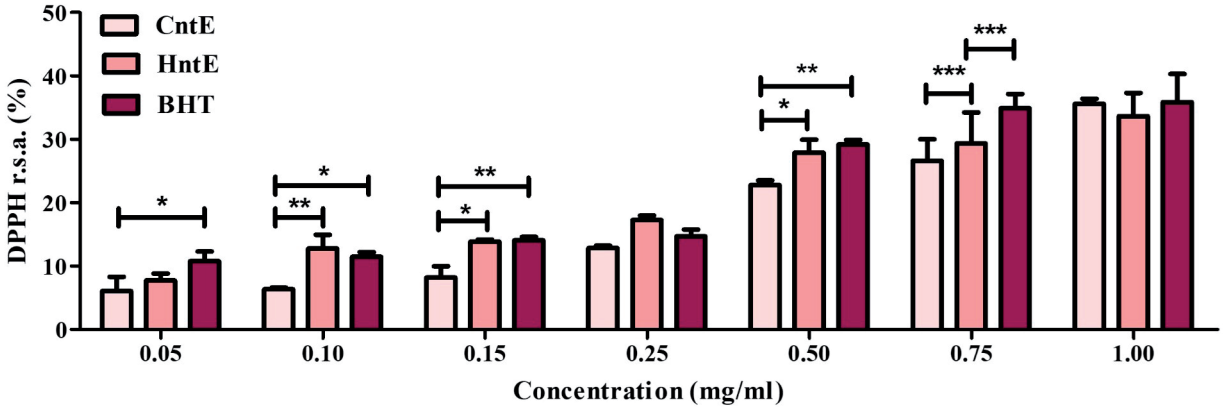


Fig. 2. Antioxidant activity of tested extracts. DPPH r.s.a.: percentage DPPH radical-scavenging activity; CntE: extract of *N. tabacum* obtained from conventional cigarettes; HntE: extract of *N. tabacum* obtained from heated tobacco products; BHT: butylated hydroxytoluene. Data were expressed as mean ± standard deviation, $n = 3$. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

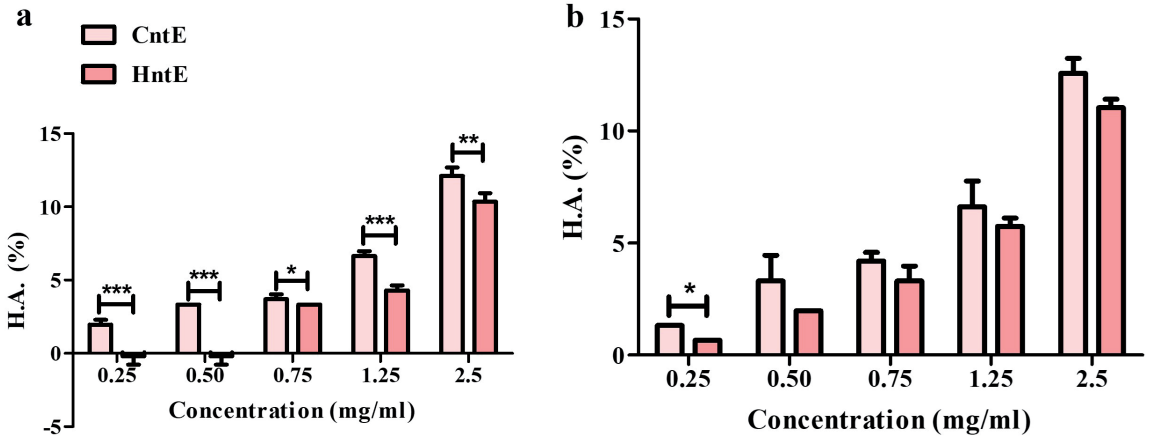


Fig. 3. *In vitro* cytotoxic activity of tested extracts. H.A.: haemolytic activity; CntE: extract of *N. tabacum* obtained from conventional cigarettes; HntE: extract of *N. tabacum* obtained from heated tobacco products; BHT: butylated hydroxytoluene. Data were expressed as mean ± standard deviation, $n = 3$. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

whereas the same concentration of CntE significantly increased the percentage of chromosomal aberrations compared with the NC ($p < 0.001$). The higher tested concentrations of both extracts (0.75 and 2.5 mg/ml) induced an increase in the percentage of chromosomal aberrations in comparison to the negative controls. Nevertheless, only CntE induced an increase in chromosomal aberrations comparable to that observed in the positive controls, i.e., bulbs treated with H_2O_2 (**Fig. 4b**).

Furthermore, detailed cytogenetic analysis, as shown in **Fig. 5a**, revealed that treatment with HntE induced a slightly higher frequency of C-mitosis compared with treatment with CntE. At the same time, CntE induced a higher frequency of fragmented chromosomes than did treatment with HntE (**Fig. 5b**). However, treatment with HntE resulted in a higher frequency of sticky chromosomes (**Fig. 5c**), chromosomal bridges (**Fig. 5d**), and multipolar mitosis (**Fig. 5e**) than treatment with CntE.

Thus, we observed that the *N. tabacum* extract obtained from conventional cigarettes exhibited pronounced genotoxic properties, which are substantially greater than those observed for the extract obtained from heated tobacco products.

Considering that the detailed cytogenetic analysis showed that the total frequency of chromosomal aberrations was substantially higher following treatment with the *N. tabacum* extract obtained from conventional cigarettes, and that fragmented chromosomes, a marker of severe chromosomal damage and genomic instability associated with cellular stress, were the most prominent aberration induced by this extract (Stevens et al., 2011), the observed genotoxicity appears to be more pronounced than that induced by HntE. In contrast, C-mitosis, a reversible chromosomal aberration (Fiskesjö, 1985), was considerably more frequent following HntE

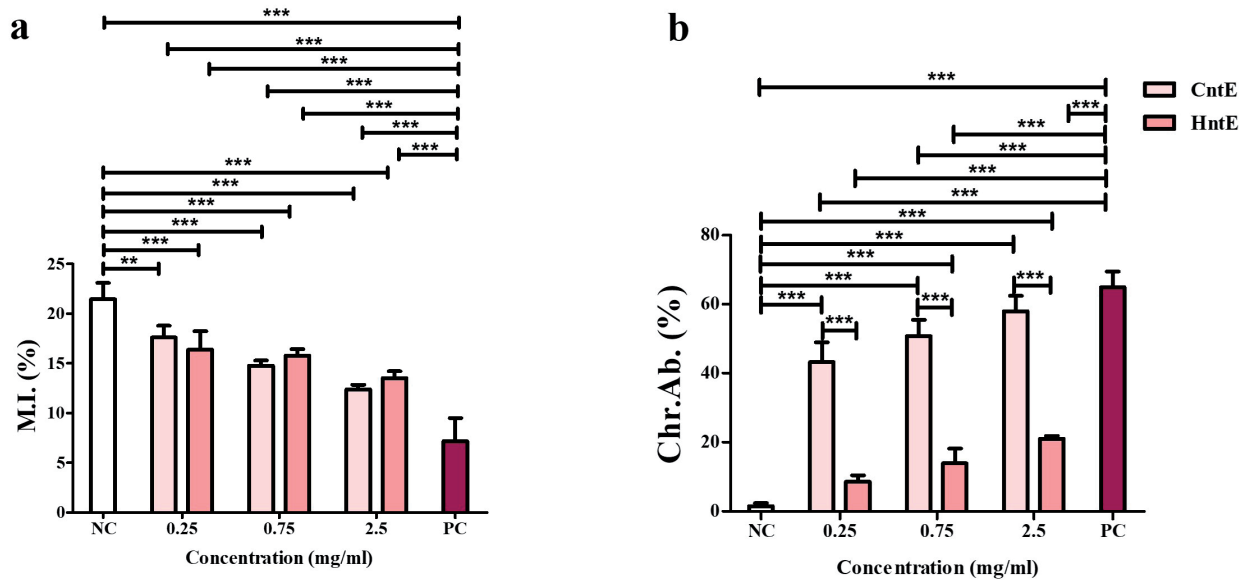


Fig. 4. *In vivo* cytotoxic and genotoxic activities of tested extracts. M.I.: mitotic index; Chr.Ab.: total percentage of cells with chromosomal aberrations; NC (negative controls): distilled water; PC (positive controls): 6% H_2O_2; CntE: extract of *N. tabacum* obtained from conventional cigarettes; HntE: extract of *N. tabacum* obtained from heated tobacco products. Data were expressed as mean ± standard deviation, $n = 5$. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

treatment than following CntE treatment. Taken together, these findings indicate that the extract obtained from conventional cigarettes exhibited greater overall genotoxic potential in the *A. cepa* test, a valuable model for assessing various genetic endpoints and predicting potential DNA-damaging effects in humans (Nefic et al., 2013).

Bearing in mind that the total phenolic and flavonoid contents of both extracts were similar, the 'Janus effect' can be excluded (Zeiger, 2003). Also, according to the manufacturers' labelling, the nicotine content was identical in both types of tobacco products. Thus, the observed differences in genotoxicity might be due to the different processing methods of tobacco. Namely, both aqueous extracts evaluated in this study were prepared using the decoction method, which is preferred when evaluating genotoxicity and other biological characteristics of plant extracts (Madić et al., 2019; Madić et al., 2021; Petrović et al., 2024; Madić et al., 2026). Moreover, tobacco used in conventional cigarettes undergoes specific conditioning and processing procedures involving additives such as ammonium salts and sugars. Some of these water-soluble compounds may readily dissolve during aqueous extraction, particularly under heating conditions, and may consequently alter the pH of the resulting extract. Changes in pH may, in turn, influence the solubility, chemical stability, and extractability of other constituents present in the tobacco matrix. This may also facilitate the release of certain potentially toxic elements, including heavy metals (Kozak & Antosiewicz, 2023; Ulutaş et al., 2026), into the aqueous extract. In addition, tobacco used in conventional cigarettes may contain tobacco-specific nitrosamines and other potentially toxic compounds formed or accumulated during curing and subsequent processing. Therefore, even in the absence of combustion, differences in the chemical composition and processing history of tobacco used for conventional and heated tobacco products may affect the composition and toxicological properties of the resulting aqueous extracts. These differences may, at least in part, explain the substantially greater genotoxic potential observed for the extract obtained from conventional cigarettes compared with that obtained from heated tobacco products (Bellosta et al., 2026).

However, the absence of manufacturer-provided data on the concentrations of tobacco-specific nitrosamines and potentially present heavy metals represents a limitation of the present study. Therefore, the proposed explanation should be interpreted with caution, as other factors may also have contributed to the observed differences. Thus, further investigation of the complete chemical profiles of both tobacco products, including the identification of the tobacco varieties used in their manufacture and consideration

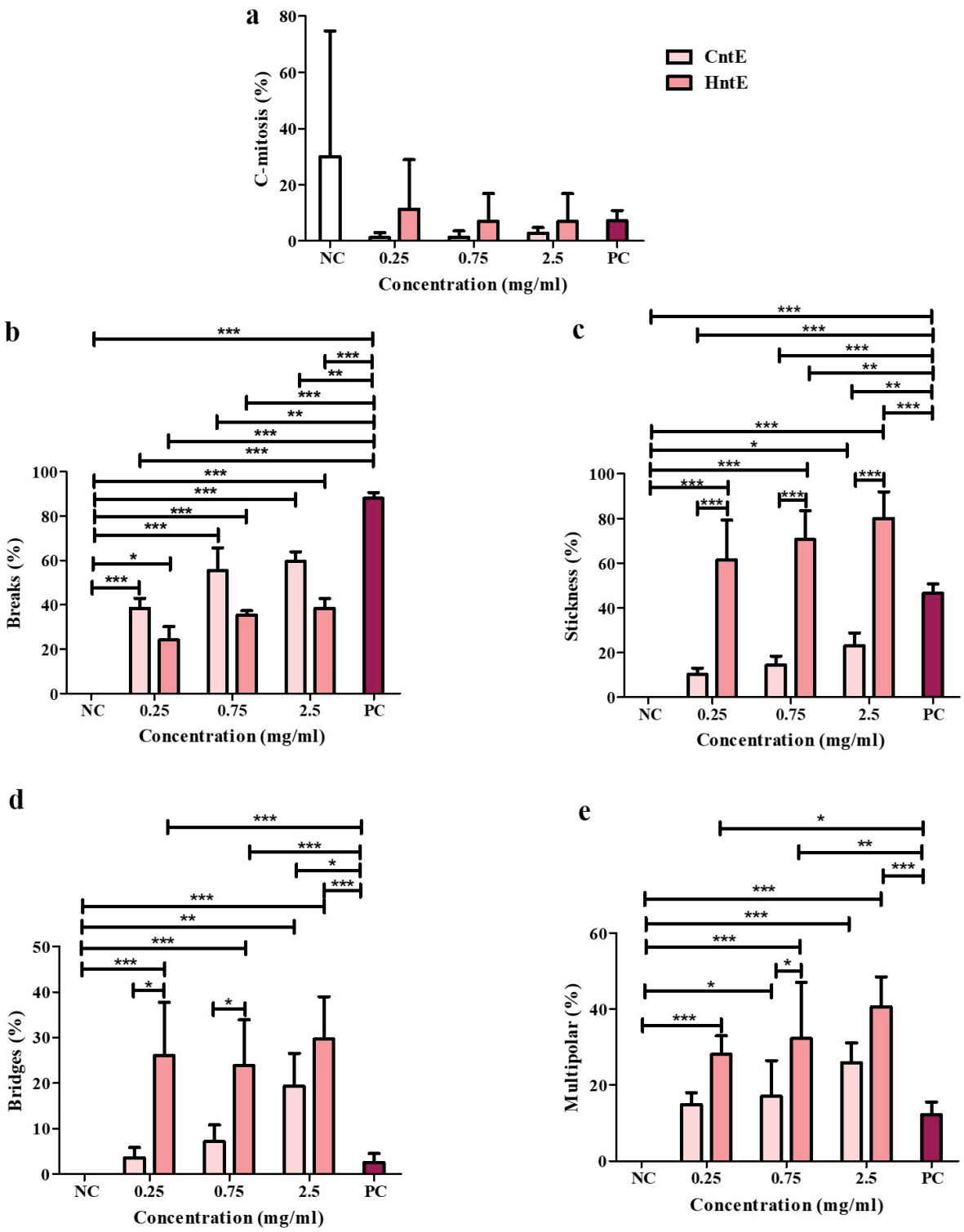


Fig. 5. Types of chromosomal aberrations in *A. cepa* meristematic cells induced by treatments. NC (negative controls): distilled water; PC (positive controls): 6% H_2O_2; CntE: extract of *N. tabacum* obtained from conventional cigarettes; HntE: extract of *N. tabacum* obtained from heated tobacco products. Data were expressed as mean ± standard deviation, $n = 5$. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

of cultivation conditions, would be valuable in future studies to provide a more comprehensive understanding of the observed effects.

Finally, despite the slightly higher antioxidant activity and lower overall genotoxic potential of the *N. tabacum* extract obtained from heated tobacco products compared with the extract obtained from conventional cigarettes, the occurrence of specific chromosomal aberrations induced, although less pronounced, still warrants attention.

Thus, we can conclude that, although the extract obtained from heated tobacco products exhibited a more favourable toxicological profile in terms of overall genotoxicity and antioxidant activity, it cannot be considered unequivocally safe. Namely, available evidence suggests reduced exposure to some harmful compounds; however, a clear reduction in health risk compared with conventional cigarettes has not yet been conclusively established.

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References

Bellosta, S., Corsini, A., & Catena, G. (2026) Heated tobacco product aerosol emission compared to cigarette smoke: A scoping review. *Toxicology Reports*, *16*, 102209. https://doi.org/10.1016/j.toxrep.2026.102209

Charlton, A. (1992) Children and tobacco. *British Journal of Cancer*, *66*(1), 1–4. https://doi.org/10.1038/bjc.1992.207

Charlton, A. (2004) Medicinal uses of tobacco in history. *Journal of the Royal Society of Medicine*, *97*(6), 292–296. https://doi.org/10.1258/jrsm.97.6.292

Duke, D., Wohlgemuth, E., Adams, K.R., Armstrong-Ingram, A., Rice, S.K., & Young, D.C. (2022) Earliest evidence for human use of tobacco in the Pleistocene Americas. *Nature Human Behaviour*, *6*(2), 183–192. https://doi.org/10.1038/s41562-021-01202-9

Fiskesjö, G. (1985) The *Allium* test as a standard in environmental monitoring. *Hereditas*, *102*(1), 99–112. https://doi.org/10.1111/j.1601-5223.1985.tb00471.x

Jankowski, M., Brożek, G.M., Lawson, J., Skoczyński, S., Majek, P., & Zejda, J.E. (2019) New ideas, old problems? Heated tobacco products – a systematic review. *International Journal of Occupational Medicine and Environmental Health*, *32*(5), 595–634. https://doi.org/10.13075/ijomeh.1896.01433

Khalili, M., Ebrahimzadeh, M.A., & Safdari, Y. (2014) Antihaemolytic activity of thirty herbal extracts in mouse red blood cells. *Archives of Industrial Hygiene and Toxicology*, *65*(4), 399–406. https://doi.org/10.2478/10004-1254-65-2014-2513

Kozak, K., & Antosiewicz, D.M. (2023) Tobacco as an efficient metal accumulator. *Biometals*, *36*(2), 351–370. https://doi.org/10.1007/s10534-022-00431-3

Leal, M., Moreno, M.A., Albornoz, P.L., Mercado, M.I., Zampini, I.C., & Isla, M.I. (2023) *Nicotiana tabacum* leaf waste: Morphological characterization and chemical-functional analysis of extracts obtained from powder leaves by using green solvents. *Molecules*, *28*(3), 1396. https://doi.org/10.3390/molecules28031396

Madić, V., Maksimović, B., Mladenović, N., Petrović, A., Manojlović, N., Jušković, M., & Vasiljević, P. (2026) Cytotoxicity and genotoxicity of *Cassia angustifolia* decoct. *Brazilian Journal of Pharmaceutical Sciences*, *62*, e24684. https://doi.org/10.1590/s2175-97902026e24684

Madić, V., Petrović, A., Jušković, M., Jugović, D., Djordjević, L., Stojanović, G., & Vasiljević, P. (2021) Polyherbal mixture ameliorates hyperglycemia, hyperlipidemia and histopathological changes of pancreas, kidney and liver in a rat model of type 1 diabetes. *Journal of Ethnopharmacology*, *265*, 113210. https://doi.org/10.1016/j.jep.2020.113210

Madić, V., Stojanović-Radić, Z., Jušković, M., Jugović, D., Popović, A.Ž., & Vasiljević, P. (2019) Genotoxic and antigenotoxic potential of herbal mixture and five medicinal plants used in ethnopharmacology. *South African Journal of Botany*, *125*, 290–297. https://doi.org/10.1016/j.sajb.2019.07.043

Mehra, R., Mohanty, V., Aswini, Y.B., Kapoor, S., & Gupta, V. (2020) Prevalence, patterns and sociocultural factors associated with use of tobacco-based dentifrices (Gul) in India. *Indian Journal of Cancer*, *57*(3), 311–320. https://doi.org/10.4103/ijc.ijc_717_18

Nefic, H., Musanovic, J., Metovic, A., & Kurteshi, K. (2013) Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by alprazolam. *Medical Archives*, *67*(6), 388–392. https://doi.org/10.5455/medarh.2013.67.388-392

Petrović, A., Madić, V., Stojanović, G., Zlatanović, I., Zlatković, B., Vasiljević, P.,

& Đorđević, L. (2024) Antidiabetic effects of polyherbal mixture made of *Centaurium erythraea*, *Cichorium intybus* and *Potentilla erecta*. *Journal of Ethnopharmacology*, *319*, 117032. https://doi.org/10.1016/j.jep.2023.117032

Ru, Q.M., Wang, L.J., Li, W.M., Wang, J.L., & Ding, Y.T. (2012) *In vitro* antioxidant properties of flavonoids and polysaccharides extract from tobacco (*Nicotiana tabacum* L.) leaves. *Molecules*, *17*(9), 11281–11291. https://doi.org/10.3390/molecules170911281

Silva, F.R. da, Erdtmann, B., Dalpiaz, T., Nunes, E., Ferraz, A., Martins, T.L.C., Dias, J.F., Rosa, D.P. da, Porawskie, M., Bona, S., & Silva, J. da (2013) Genotoxicity of *Nicotiana tabacum* leaves on *Helix aspersa*. *Genetics and Molecular Biology*, *36*(2), 269–275. https://doi.org/10.1590/s1415-47572013005000020

Stevens, J.B., Abdallah, B.Y., Liu, G., Ye, C.J., Horne, S.D., Wang, G., Savasan, S., Shekhar, M., Krawetz, S.A., Hüttemann, M., Tainsky, M.A., Wu, G.S., Xie, Y., Zhang, K., & Heng, H.H.Q. (2011) Diverse system stresses: common mechanisms of chromosome fragmentation. *Cell Death & Disease*, *2*(6), e178. https://doi.org/10.1038/cddis.2011.60

Ulutaş, K., Kosimova, S., Demir, S.N., Doğan, A., Tüfekçi, E., & Üçüncüoğlu, D. (2026) Heavy metal contamination and health risk assessment of roll-your-own tobacco in Türkiye. *Biological Trace Element Research*, *204*(7), 4745–4754. https://doi.org/10.1007/s12011-026-04992-z

World Health Organization. (2025) *WHO report on the global tobacco epidemic, 2025: warning about the dangers of tobacco. Executive summary.* Available at: https://www.who.int/publications/i/item/9789240112063

Zeiger, E. (2003) Illusions of safety: antimutagens can be mutagens, and anticarcinogens can be carcinogens. *Mutation Research/Reviews in Mutation Research*, *543*(3), 191–194. https://doi.org/10.1016/s1383-5742(02)00111-4

Zhang, W., Pan, X., Fu, J., Cheng, W., Lin, H., Zhang, W., & Huang, Z. (2024) Phytochemicals derived from *Nicotiana tabacum* L. plant contribute to pharmaceutical development. *Frontiers in Pharmacology*, *15*, 1372456. https://doi.org/10.3389/fphar.2024.1372456