ORIGINAL ARTICLE

Assessment of mutagenic effects: Combined impact of nitrogen-fixing compounds and phenol on plant and animal organisms

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ABSTRACT

Introduction: Rapid industrialisation, urbanisation, and increased use of agricultural chemicals have significantly contributed to environmental pollution, particularly affecting air, water, and soil quality. These pollutants, including nitrogen-containing compounds and phenol, pose mutagenic risks, potentially leading to genetic alterations and health issues in exposed populations. This study aims to evaluate the mutagenic potential of sodium nitrate, sodium nitrite, phenol, and their combinations on plant and animal cells.

Materials and Methods: The study utilised two experimental models: Triticum aestivum (soft wheat) and albino mice. Wheat seeds were treated with ten different solutions, including sodium nitrate (0.5%), sodium nitrite (0.5%), phenol (0.1% and 0.01%), and their combinations. Chromosomal aberrations in the wheat root tips were assessed using the acetocarmine staining method. For the animal model, albino mice were divided into control and experimental groups, receiving varying concentrations of sodium nitrate and phenol, both individually and in combination. Bone marrow smears were analysed for chromosomal aberrations, including fragments and rings, using metaphase plates.

Results: In wheat, the combined exposure to sodium nitrate (0.5%), sodium nitrite (0.5%), and phenol (0.1%) caused a significant increase in genetic alterations compared to individual treatments, with a mutation frequency 4.5 times higher than the control. In albino mice, combined exposure to high doses of phenol and nitrates induced cytogenetic changes, with the mutation frequency reaching 12.7%—1.5 times higher than the control group. Individual exposures to phenol and nitrates did not produce statistically significant mutations compared to controls.

Conclusion: The combination of phenol, sodium nitrate, and sodium nitrite had a synergistic mutagenic effect in both plants and animals, leading to more significant genetic damage than individual exposures. These findings highlight the need for careful management of environmental pollutants, as their combined impact may pose serious risks to ecological and human health. Further clinical studies are necessary to assess these effects in human populations.

KEYWORDS:
Environmental situation, atmospheric air, xenobiotics, human health, mutation, chromosome, gene

INTRODUCTION

In recent decades, rapid industrialisation, urbanisation, and the extensive use of agricultural chemicals have severely impacted the natural environment, contributing to the pollution of water, air, and food products. These environmental contaminants are known to have harmful effects on human health, particularly in vulnerable populations such as pregnant women and children.[1] Studies have shown that environmental pollution is strongly associated with various health issues, including respiratory diseases, developmental disorders, and genetic mutations.[2,3]

Children, in particular, are highly susceptible to environmental pollutants due to their developing bodies and faster metabolism, which can enhance the toxic effects of these substances.[14] Pregnant women exposed to high levels of pollutants are at increased risk of complications such as spontaneous abortions, stillbirths, and foetal developmental abnormalities, including genetic disorders.[5] Moreover, pollutants that cross the placental barrier can disrupt normal foetal development, leading to both immediate and long-term health consequences for the child.[6]

Among the many environmental pollutants, nitrogen-containing compounds (such as nitrates and nitrites) and phenol have been identified as particularly harmful.[7,8] Nitrogen oxides, even in low concentrations, have been shown to induce mutagenic effects, contributing to chromosomal aberrations and other genetic alterations.[9] Phenol, a common industrial by-product, is also recognised for its cytotoxic and genotoxic properties, especially when combined with nitrogen compounds.[10] Exposure to these chemicals has been linked to increased incidences of cancer, reproductive issues, and other genetic diseases in both human and animal models.[11,12]

Despite growing evidence of the harmful effects of nitrogen-containing compounds and phenol, there is limited research on the mutagenic effects of these substances when combined. The synergistic effects of these pollutants may exacerbate their individual toxicity, posing even greater risks to human

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health.[13-15] Understanding the genetic impacts of such combined exposure is essential for assessing environmental risks and implementing protective measures.

Therefore, this study aims to evaluate the mutagenic effects of nitrogen-containing compounds and phenol, both individually and in combination, using laboratory models. By analysing the chromosomal aberrations in plant and animal cells, this research will provide valuable insights into the genetic risks posed by these environmental toxins, informing future strategies for reducing their harmful impacts on human health.

MATERIALS AND METHODS

This study investigates the mutagenic potential of nitrogen-containing compounds and phenol, using both plant and animal test systems. Wheat cells were selected as a plant-based model due to their well-established use in environmental mutagenicity assessments, particularly in evaluating soil and water pollutants. Animal cells (albino mice) were chosen to provide a complementary model for assessing potential mutagenic risks in mammals, allowing the study to capture both environmental and physiological impacts. Combining these two experimental models provides a more comprehensive understanding of mutagenic risks across different biological systems, enhancing the robustness of the results.

Plant-Based Experiment: Wheat (Triticum aestivum)

Objective: To assess the mutagenic effects of sodium nitrate ($NaNO_3$), sodium nitrite ($NaNO_2$), phenol, and their combinations on soft wheat (Triticum aestivum) through chromosomal aberration analysis.

The experiment followed the A.K. Ergashev's protocol, developed by OECD Guidelines for the Testing of Chemicals (2016)[16], which is widely used for assessing chemical-induced chromosomal aberrations in plants. The ten chemical solutions used in this experiment were as follows:

Chemical Solutions:

1. Solution 1 : Control (pure water).
2. Solution 2 : Sodium nitrate (0.5% solution).
3. Solution 3 : Sodium nitrite (0.5% solution).
4. Solution 4 : Sodium nitrate + sodium nitrite (1:1, 0.5% solution).
5. Solution 5 : Phenol (0.1% solution).
6. Solution 6 : Phenol (0.01% solution).
7. Solution 7 : Phenol + sodium nitrate (0.1%, 0.5%, 1:1 ratio).
8. Solution 8 : Phenol + sodium nitrate (0.01%, 0.5%, 1:1 ratio).
9. Solution 9 : Phenol + sodium nitrate + sodium nitrite (0.1%, 0.5%, 0.5%, 1:1:1 ratio).
10. Solution 10: Phenol + sodium nitrate + sodium nitrite (0.01%, 0.5%, 0.5%, 1:1:1 ratio).

Seed Treatment: Twenty wheat seeds per group were frozen for 4 hours, then dried on filter paper and in a desiccator with potassium hydroxide for 4 days. After drying, the seeds were soaked in water for 48 hours to recover.

Microscopic Preparation: Root tips (5-10 mm) were fixed in acetic alcohol (3 parts ethanol, 1 part acetic acid) and stored in a refrigerator. Smears were prepared by staining root tips with acetocarmine and hydrochloric acid, followed by microscopic analysis to detect chromosomal aberrations.

Animal-Based Experiment: Albino White Mice

Objective: To assess chromosomal aberrations in the bone marrow cells of albino white mice (Mus musculus), exposed to sodium nitrate, phenol, and their combinations at various concentrations.

Type of Mice Used: Albino white mice (Mus musculus), aged 6–8 weeks, weighing approximately 20 grams, were selected for this study. The use of this specific strain is common in mutagenicity research due to their well-documented sensitivity to environmental toxins and ease of genetic monitoring.

The study followed the Guide for the Care and Use of Laboratory Animals (8 ed.) by The National Research Council (NRC) of the United States (2011)[17]. The administered dosages were derived from the maximum permissible concentration (MPC) for humans and adjusted according to the body weight of the mice. These dosages were:

- Sodium Nitrate: (MPC: 2.6 mg, Half MPC: 1.3 mg, 10x MPC: 26 mg).
- Phenol: (MPC: 0.06 mg, Half MPC: 0.03 mg, 10x MPC: 0.6 mg).

Experimental Groups:

Group I: Administered half of the permissible concentration.

- Group I-A: Control group (no chemical exposure).
- Group I-B: Exposed to phenol solution.
- Group I-C: Exposed to nitrate solution.
- Group I-D: Exposed to a combination of phenol, nitrate, and nitrite.

Group II: Administered dosages exceeding the permissible concentration by tenfold, with the same subgroup structure as Group I.

Each group included a minimum of 15 mice. Chemical solutions were administered via oral gavage.

Microscopic Examination: Bone marrow smears were prepared, and chromosomal aberrations were analysed in 2,600 metaphase plates. Chromosomal abnormalities such as dicentric chromosomes, ring chromosomes, and fragments were recorded.

Statistical Analysis: To control for potential confounders and ensure the robustness of the findings, we employed a multivariate analysis approach. The data were analysed using relative and mean values, and correlation analysis was performed to assess the strength of the relationship between chemical exposure and chromosomal damage. Additionally, subgroup analysis was conducted to evaluate potential variability within the different experimental groups.

A dose-response analysis was performed to evaluate how the severity of genetic alterations varied with different exposure

Table I: Results of Studying the Impact of Xenobiotics on Plants

Experimental Variations	Seed Germination %	Number of Anaphase Cells	Total Genetic Alterations Count		Differences Among Solutions in Terms of Genetic Variability				p-value
			Abs.	M±m%	N1	N2	N3	N5	
Solution N1	100	166	3	1,8±1,0	X	-	-	-	p>0,05
Solution N2	100	179	17	9,5+2,2	3,2	X	-	-	p>0,05
Solution N3	95	180	23	12,8+2,5	4,1	-	X	-	p>0,05
Solution N4	95	153	21	13,7±2,8	4,0	-	-	-	p>0,05
Solution N5	60	86	3	3,5+1,9	0,8	-	-	X	p>0,05
Solution N6	70	178	18	10,1+2,3	3,4	-	-	-	p>0,05
Solution N7	95	170	25	14,7±2,7	4,4	1,7	1,5	-	p>0,05
Solution N8	80	142	24	16,9+3,1	4,6	1,0	1,7	-	p>0,05
Solution N9	95	172	34	19,6+3,0	5,8	2,7	2,0	4,5	p>0,05
Solution N10	100	231	33	14,3±2,3	5,0	1,5	0,4	1,3	p>0,05

levels. This allowed us to determine whether increasing the dosage of the chemicals resulted in a proportional increase in chromosomal aberrations. The analysis helped establish the correlation between dose and mutagenic effect, contributing to the assessment of safe exposure levels.

Sensitivity Analysis: Sensitivity analysis was conducted by adjusting key variables in the statistical model to ensure the results were robust across a range of assumptions.

Randomisation and Blinding

Randomisation was not used due to the controlled laboratory conditions and standardized exposure protocols. Blinding was not applicable as outcome assessment was based on objective cytogenetic criteria.

Ethical Statement for Animal Experiments

All animal procedures were carried out in accordance with the ethical standards approved by the Animal Ethics Committee of Ministry of Health of the Republic of Uzbekistan and Committee for Veterinary and Livestock Development of the Republic of Uzbekistan, following the Declaration of Helsinki and relevant national legislation. The protocol was reviewed and approved under protocol number 7/25-1953, issued by the Special Ethical Committee of MOH (SEC).

RESULTS

Mutagenic alterations in anaphase cells in the prepared smears were observed and analysed under the microscope. The research results concerning xenobiotic levels in higher plants demonstrated that upon exposure to pure water (N1), a 0.5% solution of sodium nitrate (N2), and a solution of 0.1% phenol with 0.5% sodium nitrate and 0.5% sodium nitrite (N9), 100% of the seeds from the solutions germinated. However, seeds exposed to solutions N5 (60%), N6 (70%), and N8 (80%) displayed the lowest percentage of growth. In the remaining four solutions, seed growth reached 95%.

From the germinated root tips of the plants (wheat), 85 smears were prepared for microscopic examination, totalling 1,656 anaphase cells analysed. Throughout the experiments, isolated and paired chromosome fragments, chromosomes, and chromatic bridges were detected. The absence of micronuclei in chromosomes and similar cellular anomalies were grouped with other types of mutations. The experiments demonstrated that harmful chemical compounds in the external environment exhibit mutagenic activity on plant organisms.

The frequency of spontaneous mutations in the control group experiments was 1.8%. Under the influence of 0.5% nitrogen-fixing compounds, the mutation frequency increased by 5.7 to 7 times compared to the control group. The comparison group differed from the experimental observations, although the magnitude of genetic changes was not statistically significant. The germination of plant seeds decreased by 0.1% with individual exposure to a 0.1% phenol concentration. In our opinion, this phenomenon is likely associated with the death of plant cells. Reducing the impact of phenol by tenfold (to 0.01%) increased the number of genetically modified cells by five times compared to the control group.

With the combined influence of nitrogen-fixing compounds (at a concentration of 0.5%) and a 0.01% phenol solution, the frequency of mutation occurrences significantly increased compared to the control group. The results obtained from individual exposure to xenobiotics did not lead to statistically significant changes in all cases (Table I).

It is noteworthy that the combined exposure of 0.1% phenol with high concentrations of sodium nitrate and sodium nitrite (0.5% solutions) led to more profound genetic alterations not only in the control group but also in their separate exposures. Such alterations were observed to be 2.7 times greater than the impact of nitrates alone, 2 times greater than the effect of nitrites alone, and 4.5 times greater than the influence of 0.1% phenol. The outcomes of these experiments provide the opportunity to identify combinations of xenobiotics that yield more potent and effective effects amidst the various combinations tested.

The experiments following Method 2 commenced with the administration of high doses of substances to the experimental animals (more than ten times the MPC).

As shown in Table II, the frequency of spontaneous mutations in the comparison group was relatively low, at 8.7 ± 1.3%, as substantiated by the analysis of metaphase plates.

The effects of the studied chemical substances at doses exceeding the MPC by tenfold vary depending on their application. No statistically significant changes in mutational processes were observed upon separate exposure

Table II: Results of Investigating the Impact of High Doses of Xenobiotics on Animals

Experimental variations	Number of metaphase plates	Spectrum of aberrations					Cytogenetic changes		Differences among experiments in terms of genetic variability			p-value
		Isolated fragments	Paired fragments	Dicentric chromosome	Acentric chromosome	Centric ring	Abs.	M±m%	N1	N2	N3	
N1 - control animals	496	22	7	10	2	2	43	8,7±1,3	X	-	-	-
N2 - experiment-1 phenol	329	15	2	10	2	0	29	8,8±2,0	-	X	-	p>0,05
N3 - experiment-2 nitrate	163	7	1	5	2	0	15	9,2±2,3	-	-	X	p>0,05
N4 - experiment-3 phenol-nitrate	831	35	25	36	8	2	106	12,7±1,2	2,4	2,0	1,3	p<0,01

Table III: Results of Investigating the Impact of Low Doses of Xenobiotics on Animals

Experimental variations	Number of metaphase plates	Spectrum of aberrations					Cytogenetic changes		Differences among experiments in terms of genetic variability			p-value
		Isolated fragments	Paired fragments	Dicentric chromosome	Acentric chromosome	Centric ring	Abs.	M±m%	N1	N2	N3	
N1 - control animals	496	22	7	10	2	2	43	8,7±1,3	X	-	-	
N2 - experiment-1 phenol	57	2	1	2	1	0	6	9,0±3,4	-	X	-	p>0,05
N3 - experiment-2 nitrate	441	17	11	23	2	8	61	13,8±1,6	-	-	X	p<0,01
N4 - experiment-3 phenol-nitrate	275	12	12	14	0	5	43	15,6±2,2	2,8	2,1	0,6	p<0,01

to nitrates and phenol (8.8 ± 2.0% and 9.2 ± 2.3%, respectively, with $p > 0.05$). However, with the combined action of nitrates and phenol at the same doses, genetic alterations significantly intensified compared to the control group, reaching 12.7 ± 1.2% (1.5 times higher). Changes resulting from separate exposure to nitrites and nitrates did not significantly differ statistically from each other.

The obtained results provide grounds to conclude that high doses of phenol and nitrates exhibit cytotoxic effects and suppress genetic alterations. Meanwhile, it is confirmed that a stronger manifestation of genetic changes in plants occurs with the combined influence of chemical agents.

Upon exposure to the preparations under study at concentrations half of the MPC, the following results were observed (Table III).

As evident from Table III, the number of mutated cells and identified mutations upon exposure to phenol at 0.5 MPC (individually) amounted to 9.0 ± 3.4%. This result is not statistically significant when compared to the control group ($p > 0.05$). The total number of genetic alterations when exposed to a sodium nitrate solution concentration two times less than the MPC was 13.8 ± 1.6%, which is 1.6 times higher than the control group ($p < 0.01$). With the combined influence of sodium nitrate and phenol, the level of genetic alterations increased by 1.8 times compared to the control group, reaching 15.6 ± 2.2% ($p < 0.01$).

DISCUSSION AND CONCLUSION

The results of our study highlight the mutagenic potential of phenols and nitrates, as evidenced by the chromosomal aberrations observed in both wheat and albino mice models.[18,19] However, it is crucial to carefully consider how these findings may relate to human health. While the mutagenic effects identified in plant and animal models suggest a potential risk for humans, direct extrapolation must be approached with caution. Biological responses to chemical exposures can vary significantly between species due to differences in metabolism, physiology, and genetic makeup.[20] Therefore, further clinical studies are essential to accurately assess the risks these compounds pose to human health, particularly with regard to potential carcinogenic effects and genetic damage.

Additionally, potential sources of bias, such as environmental variability and interspecies differences, must be acknowledged. Factors such as variations in environmental conditions, genetic diversity among test subjects, and differences in exposure levels can influence the outcomes of mutagenicity studies.[21] These variables may affect the robustness and generalisability of our findings, underscoring the need for a comprehensive assessment that includes a range of environmental conditions and a broader selection of biological models.

Moreover, while our study focused on chromosomal aberrations, it is important to delve deeper into the underlying mechanisms driving these genetic changes. Understanding processes such as DNA repair pathways, specific gene mutations, and the molecular interactions of phenols and nitrates with genetic material is crucial for elucidating how these compounds exert their mutagenic effects. Insights into these mechanisms can enhance our understanding of the biological impact of chemical exposures and help inform strategies for mitigating their potential risks.

In conclusion, while our findings contribute valuable data regarding the mutagenic potential of phenols and nitrates, further research is necessary to explore their implications for human health, address potential biases, and investigate the molecular mechanisms underlying the observed genetic alterations. A comprehensive approach will provide a clearer understanding of the risks associated with these compounds and inform public health guidelines.

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