Original Article
DOI: https://doi.org/10.70749/ijbr.v3i7.2121

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Evaluation of DNA Damage in Human Lymphocytes Exposed to Triclosan by Alkaline Comet Assay

Waqas Ali[1], Majid Ullah[1], Abdullah Khan[1], Laiba Zahoor[1], Aqsa Zahid[1], Eman Afrooz[1], Syeda Sawaiba Ali[1], Asma Hussain[1], Muhammad Shoaib[1], Aiman Mehroon[1], Momin Shah[1]

[1]Institute of Zoological Sciences, University of Peshawar, Peshawar, Pakistan

ARTICLE INFO

Keywords: Triclosan, genotoxicity, antimicrobial agent, lymphocytes

Correspondence to: Waqas Ali,
Institute of Zoological Sciences, University of Peshawar, Peshawar, Pakistan
Email: waqasaliuop@gmail.com

Declaration

Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History

Received: 02-05-2025 Revised: 03-07-2025
Accepted: 07-07-2025 Published: 30-07-2025

ABSTRACT

In the present study, the in-vitro effect of Triclosan was tested on human peripheral lymphocytes taken in different concentrations. The genotoxicity of these antimicrobial agents was assessed using alkaline comet assay. The lymphocytes were isolated from the blood of non-smoker healthy students, which were treated with different concentrations of the selected antimicrobial agent i.e. Triclosan (2.5mg/mL, 4mg/mL, 5mg/mL, 6.5mg/mL, 7.5mg/Ml, 10mg/mL) and were kept for 1 hour in a dry bath incubator. The result demonstrated that Triclosan was able to induce DNA damage with all concentrations. The amount of damage was directly proportional to the concentration of antimicrobial drugs used.

INTRODUCTION

Antimicrobial agents are molecules that either eradicate or restrict the replication of viruses, bacteria, fungus, and parasitic organisms, and other microbes. A natural, semi-synthetic, or synthetic material that holds antimicrobial property (eliminates or restricts the spread of microbes) at concentrations obtainable in vivo is usually referred to as an antimicrobial substance (292).

The kind of antibacterial substance and the type of bacteria determine their modes of impact. The peptidoglycan-based stiff cell wall of numerous bacteria is crucial to their existence and morphology. Antimicrobial substances aimed at the enzymes engaged in peptidoglycan formation, such as beta-lactams (e.g., penicillins, cephalosporins) and glycopeptides (e.g., vancomycin), cause lysis of cells and their demise (1)

For instance, penicillin destroys the bacterial cell wall and induces osmotic damage by inhibiting transpeptidase, which stops peptidoglycan chains from linking together. Certain antimicrobial compounds cause microbial cellular membranes to break down, which results in intracellular fluid leaking out and dying cells. For instance, Polymyxins, such as colistin, increase permeability of membranes by binding to the lipopolysaccharides of Gram-negative bacterial exterior membrane. Fungus cell membranes contain ergo sterol, which antifungal drugs like amphotericin B bind to create holes that compromise the stability of the membrane (2).

The development and spread of microorganisms depend on the manufacturing of proteins. Antimicrobials that attack ribosomes stop protein molecules from being generated. Tetracyclines stop tRNA from attaching to the ribosome, which stops the protein chain from lengthening. In the process of the manufacture of proteins, macrolides, such as erythromycin, interact with the 50S subunit of the ribosome and prevent transportation. When aminoglycosides, such gentamicin, attach to the 30S subunit, mRNA is misinterpreted and inactive peptides are produced (3).

Table 1

Types	Effect / Uses	Citation
Anti-bacterial	Uses against bacteria	Leekha, Terrell, & Edson, 2011
Anti-viral	Uses against viruses	
Anti-fungal	Uses against fungi	
Anti-parasitic	Uses against other parasitic organisms	

Types of Antimicrobial Agents

Describes the effects of antibiotic resistance as a recurring worldwide threat to health and emphasizes initiatives to address this intricate issue. To slow the rise in antibiotic resistance, legislative initiatives, laws, treatment advancement, and dissemination of knowledge are crucial. In a time when there are few novel medicines for bacterial diseases, the challenging endeavor of reducing antibiotic resistance falls to investigators physicians, and legislators. Antibiotic resistance in human beings and livestock industries may be addressed, according to the researchers, by legislation, tracking, inspection of practices, and innovative therapies. The intricacy of antibiotic resistance is emphasized in this paper, along with the necessity of a multimodal strategy to enhance health care results (4).

The primary objective of this study is to evaluate the in-vitro genotoxic effects of Triclosan on human lymphocytes using the Comet Assay, focusing on assessing the extent of DNA damage. Furthermore, the study aims to compare the level of DNA damage across different concentrations of Triclosan to determine whether a dose-dependent relationship exists between Triclosan exposure and the degree of DNA damage. To ensure the reliability and validity of the results, the collected data will be statistically analyzed using appropriate methods, including Analysis of Variance (ANOVA), to evaluate the significance of observed differences among treatment groups.

Triclosan (TCS; 5-chloro-2-(2,4-dichlorophenoxy) phenol) is a chlorinated phenolic antimicrobial that has been widely used in consumer and healthcare products (toothpastes, soaps, plastics) and is frequently detected in environmental matrices and biological samples. Concern about human health effects has grown because TCS is persistent in the environment, can bioaccumulate to some extent, and has been associated with endocrine, oxidative-stress and other molecular effects in mammalian systems (5)

The alkaline single-cell gel electrophoresis (comet) assay is a sensitive method for detecting primary DNA damage (single- and double-strand breaks and alkali-labile sites) in individual cells. Its adaptability to peripheral blood cells makes it particularly useful for human biomonitoring and toxicology studies intended to evaluate genotoxic exposures in occupational or environmental contexts. Standardized protocols and consensus recommendations have improved comparability across studies, but careful control for cytotoxicity, sample handling, and scoring criteria remains essential because those factors strongly affect comet outcomes (6)

Direct studies using lymphocytes (the most relevant target for the present study design) are fewer but informative. Experimental work in rodent lymphocytes has shown TCS-induced increases in comet parameters (olive tail moment/percent tail DNA) at concentrations that also raised oxidative stress markers; importantly, antioxidant cotreatments (e.g., N-acetylcysteine, curcumin, resveratrol) attenuated the DNA damage, supporting oxidative mechanisms. These lymphocyte data indicate that peripheral blood cells are responsive to TCS-induced DNA strand breaks and are therefore an appropriate cell type for human biomonitoring using the alkaline comet assay (7).

MATERIALS AND METHODS

The present study was conducted to assess DNA damage in human lymphocytes exposed to various concentrations of Triclosan using the Comet Assay. Blood samples were randomly collected from healthy students of the Institute of Zoological Sciences, University of Peshawar, through venipuncture. The single-cell gel electrophoresis (SCGE) or microgel technique, originally developed by Singh et al.(8), was employed under alkaline conditions (pH 13) to detect single-strand breaks and alkali-labile lesions in individual cells. This method has been extensively used in studies of oxidative DNA damage, genotoxicity, radiation exposure, pollution, and aging (9). Triclosan and various reagents including phosphate buffer saline (PBS), normal and low melting agarose, ethylenediamine tetraacetic acid (EDTA), dimethyl sulfoxide ($DMSO_4$), trichloroacetic acid, and sodium chloride were utilized to prepare solutions such as agarose gels, lysing buffer, electrophoresis buffer, neutralization buffer, and phosphate buffer. Essential laboratory materials included microcentrifuge tubes, Coplin jars, microscope slides, micropipettes, centrifuge machines, and electrophoresis apparatus. Approximately 3 mL of peripheral blood was drawn into EDTA tubes, and lymphocytes were separated by layering the blood over lymphocyte separation medium and centrifuging at 4000 RPM for 30 minutes, resulting in distinct plasma, buffy coat, and red blood cell layers. The lymphocyte-rich buffy coat was isolated, washed with PBS, and suspended to form a homogeneous cell mixture. The isolated lymphocytes were then treated with varying concentrations of Triclosan and incubated at 37°C for one hour. For the preparation of cell microgels, slides were sequentially coated with three agarose layers—first with 1.5% normal melting agarose for pre-coating, followed by two layers of 0.5% low melting agarose containing lymphocytes, each solidified on ice. The slides were then incubated in chilled lysing solution at 4°C for two hours to lyse cells, followed by electrophoresis in alkaline buffer (pH > 13) at 25 V and 300 mA for 25 minutes to allow DNA

migration. Slides were neutralized thrice using Tris buffer (pH 7.5), dehydrated with absolute ethanol, air-dried, rehydrated in chilled distilled water, and stained with acridine orange for fluorescence microscopy. One hundred randomly selected cells per slide were scored under the fluorescence microscope to evaluate tail length and DNA migration, serving as indicators of DNA strand breaks. Based on the extent of tail formation, comets were classified into five categories ranging from Class 0 (no damage) to Class 4 (maximum damage), providing a quantitative measure of Triclosan-induced DNA damage.

Table 2

The following five comet classes were made on the basis of comet tail length observation.

Comet class	Tail length	Description
Class 0	No Tail length	No DNA damage
Class 1	1.5 times the diameter of comet nucleus	Slight DNA damage
Class 2	1.5 to 2 times the diameter of comet nucleus	Moderate DNA damage
Class 3	2 to 2.5 times the diameter of comet nucleus	Highly DNA damage
Class 4	Totally change	Maximum DNA damage

RESULTS

This study was performed in order to evaluate the effects of Triclosan on the DNA of human lymphocytes. Blood samples were collected from healthy students of the Institute of Zoological Sciences, University of Peshawar. Lymphocytes were isolated from the blood and treated with different concentrations of Triclosan. The DNA was visualized under fluorescence microscope by treating them with fluorescent stains. The final analysis was done manually by counting the comet score from the images taken as a result of fluorescence microscopy of comet assay slides. Damaged DNA cells were figured by comet appearance with a brightly colored head and tail to one side formed by the DNA containing strand breaks that run away during electrophoresis. One hundred cells in each sample were analyzed to get the comet score.

As blood was taken from healthy students, so there were no comets during analysis of microgel containing cells of control group (negative control). High amount of DNA was observed in the cells of positive control group due to the toxicity is caused by H2O2. The DNA damage in both control groups is shown is the table.

Table 3

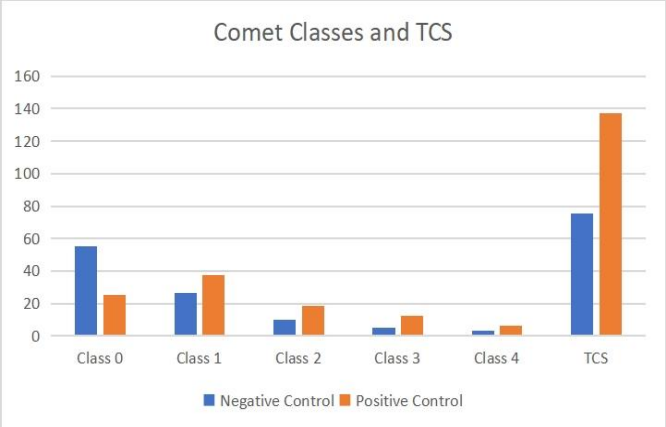
Comet classes and TCS of Control (positive and negative) comet (Per 100 cells analyzed)

Concentration	Class 0	Class 1	Class 2	Class 3	Class 4	TCS
Negative control	55.3 ± 8.7	26.3 ± 7.2	10.0 ± 2.0	5.0 ± 1.0	3.3 ± 1.5	75.7 ±12.6
Positive Control	25.3 ± 5.7	37.3 ± 6.7	18.3 ± 10.7	12.7 ± 3.2	6.3 ± 1.5	137.3 ± 18.5

Significant difference between the control groups, p = 0.009 (t- test)

Figure 1.

DNA breaks induced by Triclosan



Cells were treated with different concentrations of the selected antimicrobial agent i.e, Triclosan and incubated for 1 hour. Six different concentrations were used during the study to analyze the DNA damage. It was found that genotoxicity increases with increase in concentration of Triclosan. It reveals that increase amount of Triclosan can cause more DNA damage.

DNA damage induced by different concentrations of Triclosan:

At lower concentration, the DNA damage observed was less than the other concentrations of Triclosan. The DNA damage was maximum at 10mg / ml and minimum at 2.5 mg / ml.

Table 4

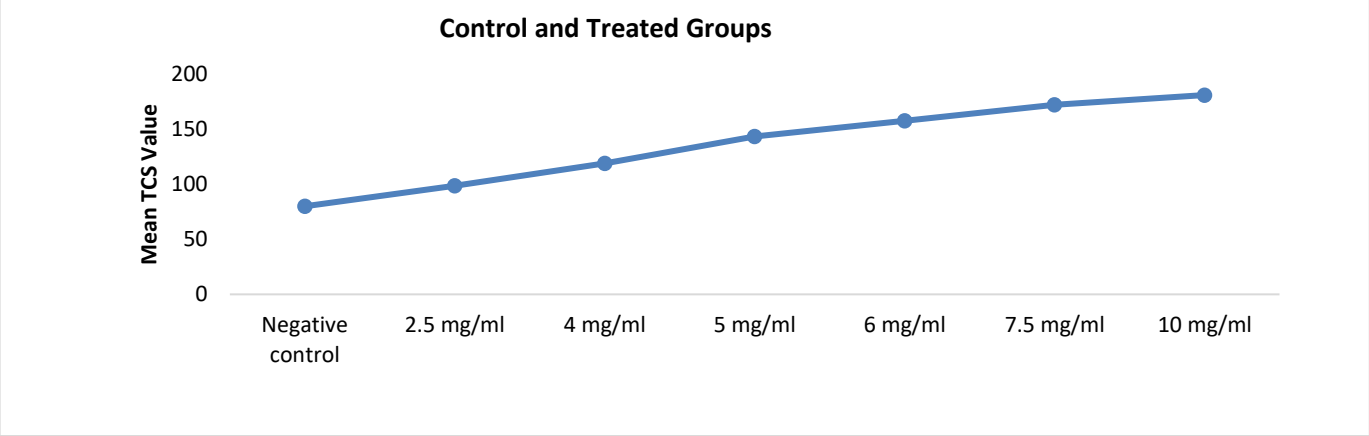
Comet classes and TCS (mean ± SD) values in response to the in vitro treatment of human lymphocytes with Triclosan (100 cells analyzed per sample)

Concentration mg/ml	Class 0	Class 1	Class 2	Class 3	Class 4	TCS
Negative Control	**52.6 ± 3.6**	**27.2 ± 2.0**	**10.8 ± 1.9**	**6.0 ± 0.7**	**3.4 ± 1.0**	**80.0 ± 6.4**
2.5 mg/ml	**40.2 ± 4.1**	**29.4 ± 2.6**	**15.0 ± 2.2**	**9.0 ± 1.2**	**6.4 ± 1.1**	**98.6 ± 5.2**
4mg/ml	**29.6 ± 3.8**	**27.0 ± 2.4**	**18.2 ± 2.0**	**13.1 ± 1.8**	**12.1 ± 1.4**	**118.9 ± 6.1**
5mg/ml	**18.3 ± 2.9**	**24.6 ± 3.1**	**21.0 ± 3.1**	**17.5 ± 2.00**	**18.6 ± 1.6**	**143.3 ± 5.7**
6.5mg/ml	**12.8 ± 3.2**	**20.4 ± 2.7**	**22.6 ± 2.0**	**20.8 ± 1.5**	**23.4 ± 2.2**	**157.6 ± 6.3**
7.5mg/ml	**8.6 ± 2.0**	**15.7 ± 2.5**	**23.8 ± 2.2**	**23.5 ± 1.8**	**28.4 ± 2.6**	**172.3 ± 5.8**
10mg/ml	**5.4 ± 1.8**	**11.2 ± 2.1**	**25.6 ± 2.5**	**25.0 ± 2.3**	**32.8 ± 3.0**	**181.0 ± 6.1**

TCS, total comet score; SD, standard deviation, Significant difference relative to negative control, p = 0.008 (one-way ANOVA)

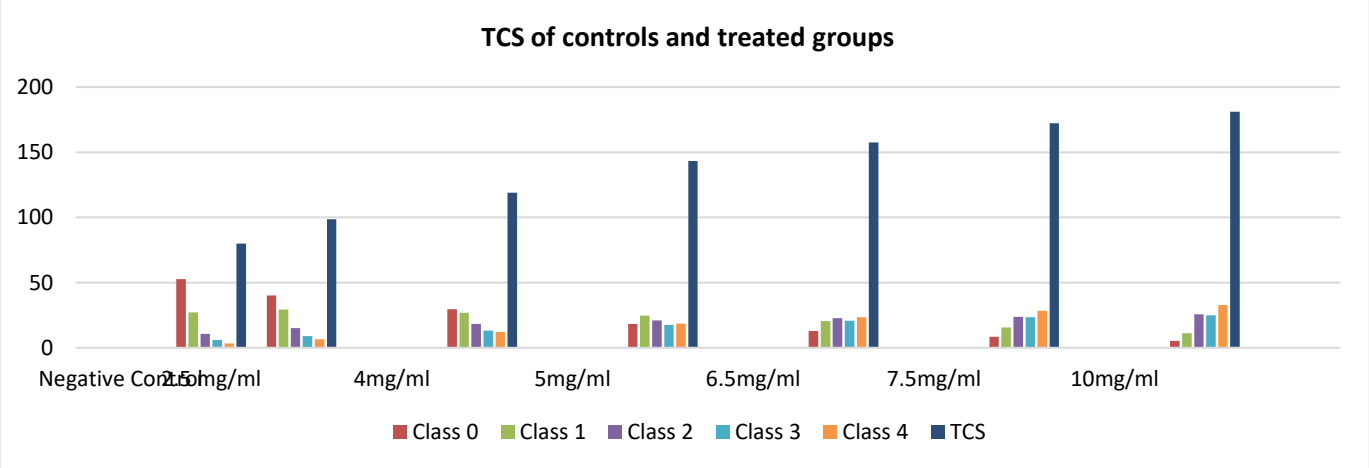
Figure 2

Mean Total Comet Score (TCS) Value



TCS of Control and Treated Group

Table 5



Class 0 (control group); NO DNA Damage

Figure 3

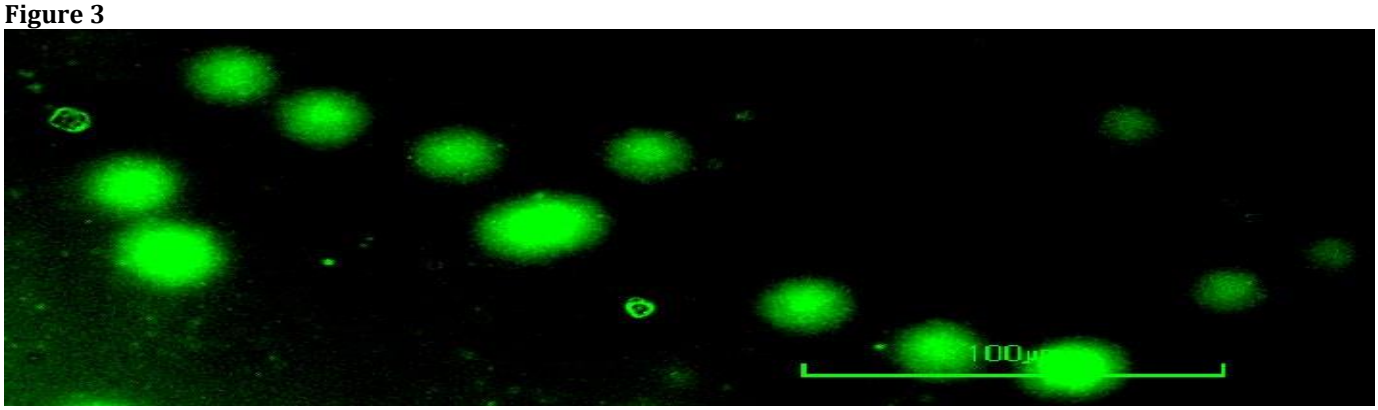
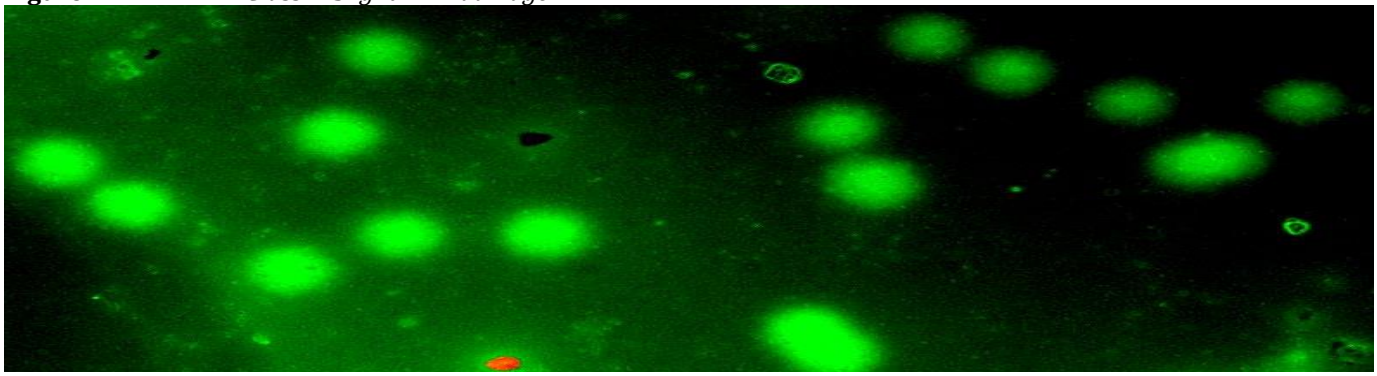


Figure 4 *Class 1. Slight DNA damage*



DISCUSSION

Our result indicates that, as the concentration of Triclosan increases the length of the DNA tail also increases. It means that the selected antimicrobial drug has genotoxic effects on healthy lymphocytes, and it causes DNA damage at all the applied concentrations. This study explored significant differences in DNA damage levels of the exposed groups and control groups. The TCS in occupationally exposed groups was greater than control group as exposed groups represented the significantly increased DNA migration, a lot of high damaged DNA spots in contrast to control group.

Two control groups were taken (positive and negative). Six different concentrations of the selected antimicrobial agent (Triclosan) were taken which were 2.5, 4, 5, 6.5, 7.5,10 mg/mL. They were introduced to human lymphocytes and incubated for 1 hour. Hundred cells in each sample were analyzed and comet scores were calculated. In our study, we observed that higher levels of DNA damage were caused by Triclosan. A high amount of DNA damage was observed in cells of the positive control group due to toxicity caused by H_2O_2. At low concentration of triclosan i.e; 2.5 mg/mL, the TCS value of DNA damage of was 98.6. Hence it is known that even at lower concentration the DNA damage is high. At of the mentioned antibiotics, moderate DNA damage was observed. At 4 mg/mL concentration of triclosan, TCS value of DNA damage was 118.9 It was found that DNA damage at 4 mg/mL is higher than 2.5 mg/mL. At 10mg/mL concentration of the selected antimicrobial agent, high level of DNA damage was observed. At this concentration, TCS value of DNA damage was 181.0. It was found that DNA damage has a direct relation with high concentration. If we increase concentration of triclosan then the DNA damage will be higher as the above statement.

Among the cellular contents, DNA is an important target of environmental stress is aquatic and terrestrial organism. The level of strand breakage in DNA has been proposed as a sensitive indicator of genotoxicity and an effective biomarker in environmental Biomonitoring (10). DNA damage may be induced directly by chemical such as H2O2, or reactive oxygen species (ROS) Genotoxicity tests as SCGE assay have become extensively valuable as biomarkers for different cells in-vitro. SCGE detects single and double-stranded breaks as well as apurinic sites (11).

In the present study, the selected antimicrobial drug induced DNA damage in human lymphocytes exposed to all concentrations evaluated. We conducted the present research to check the genotoxic effect of commonly used antimicrobial agent, Triclosan by administering it in different concentrations into human peripheral lymphocytes, incubated for 1 hour in a dry bath incubator, in-vitro. Different concentrations of Triclosan were introduced into cells to check the DNA damage in lymphocytes in-vitro. In this study, single cell gel electrophoresis method was used to detect the DNA damage in lymphocytes of healthy students. SCGE is a very simple method to detect even very low levels of DNA damage (12)

CONCLUSIONS

The sensitivity of comet assay compared to other established methods was judged during analyzing the sample which indicates its importance in determining genotoxicity affecting cell susceptibility. Most of the study indicates that overdose of the selected antimicrobial drug cause genotoxicity in various cells including macrophages and lymphocytes. In conclusion, the result from the present-day study demonstrated that the selected antimicrobial agent induced DNA damage which increased with increase in concentration of the triclosan added, as evidence of SCGE assay. Result of this investigation could provide useful data for hazard assessment related to these compounds commonly used in cosmetics.

Recommendations

Triclosan is commonly used in concentrations up to 0.3% (3 mg/mL) in products such as soaps, toothpaste, and deodorants, according to the regulations of the U.S. FDA and European Commission. But according to our research results it can cause DNA damage at 2.5mg/Ml.

Regulatory Re-evaluation: Health authorities should reconsider the continued use of triclosan in cosmetics and personal care products, especially in light of its DNA-damaging potential demonstrated in-vitro.

Public Awareness: Public awareness campaigns should be initiated to educate consumers about the potential health risks of triclosan-containing products.

Further Research: Additional in-vivo studies and long-term epidemiological investigations are needed to better understand the actual risk of triclosan exposure in humans.

Alternatives: Safer antimicrobial alternatives should be developed and promoted in cosmetic and hygiene products.

REFRENCES

1. Ciofu O, Moser C, Jensen PØ, Høiby N. Tolerance and resistance of microbial biofilms. Nature Reviews Microbiology. 2022;20(10):621-35. https://doi.org/10.1038/s41579-022-00682-4
2. Mertens RT, Gukathasan S, Arojojoye AS, Olelewe C, Awuah SG. Next generation gold drugs and probes: chemistry and biomedical applications. Chemical reviews. 2023;123(10):6612-67. https://doi.org/10.1021/acs.chemrev.2c00649
3. Luo Y, Song Y. Mechanism of antimicrobial peptides: antimicrobial, anti-inflammatory and antibiofilm activities. International journal of molecular sciences. 2021;22(21):11401. https://doi.org/10.3390/ijms222111401
4. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB, et al. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of infection and public health. 2021;14(12):1750-66. https://doi.org/10.1016/j.jiph.2021.10.020
5. Alfhili MA, Lee M-H. Triclosan: an update on biochemical and molecular mechanisms. Oxidative medicine and cellular longevity. 2019;2019(1):1607304. https://doi.org/10.1155/2019/1607304

6. Ladeira C, Møller P, Giovannelli L, Gajski G, Haveric A, Bankoglu EE, et al. The comet assay as a tool in human biomonitoring studies of environmental and occupational exposure to chemicals—a systematic scoping review. Toxics. 2024;12(4):270. https://doi.org/10.3390/toxics12040270
7. Duarte NdAA, Lima LEd, Maraslis FT, Kundi M, Nunes EA, Barcelos GRM. Acute toxicity and DNA instability induced by exposure to low doses of triclosan and phthalate DEHP, and their combinations, in vitro. Frontiers in genetics. 2021;12:649845. https://doi.org/10.3389/fgene.2021.649845
8. Gonçalves S, Gaivão I. Natural ingredients common in the Trás-Os-Montes region (Portugal) for use in the cosmetic industry: a review about chemical composition and antigenotoxic properties. Molecules. 2021;26(17):5255. https://doi.org/10.3390/molecules26175255
9. Arenaz P. Chinese hamster apurinic/apyrimidinic endonuclease (chAPE1) expressed in sf9 cells reveals that its endonuclease activity is regulated by phosphorylation. The FEBS Journal. 2025. https://doi.org/10.1111/j.1742-4658.2010.07879.x
10. Jiang N, Naz S, Ma Y, Ullah Q, Khan MZ, Wang J, et al. An overview of comet assay application for detecting DNA damage in aquatic animals. Agriculture. 2023;13(3):623. https://doi.org/10.3390/agriculture13030623
11. Gupta P, Makkar TK, Goel L, Pahuja M. Role of inflammation and oxidative stress in chemotherapy-induced neurotoxicity. Immunologic Research. 2022;70(6):725-41. https://doi.org/10.1007/s12026-022-09307-7
12. Muruzábal-Gambarte D. Optimization of the in vitro comet assay as a tool for mechanistic risk assessment. 2021.