GENOTOXIC EFFECTS OF VEHICULAR EMISSIONS ON THE DNA OF BUCCAL CELLS IN DRIVERS AT CENTRAL PARK , SAPELE ROAD , BENIN CITY : A MICRONUCLEI ASSAY -BASED STUDY

BY

GIFT UGWUDIKE (MISS)

LSC 2009816

UNIVERSITY OF BENIN

BENIN CITY

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

CERTIFICATION

This is to certify that this project titled **"GENOTOXIC EFFECTS OF VEHICULAR EMISSIONS ON THE DNA OF BUCCAL CELLS IN DRIVERS AT CENTRAL PARK, SAPELE ROAD, BENIN CITY : A MICRONUCLEI ASSAY -BASED STUDY"** was carried out by **Gift UGWUDIKE (MISS)** with Matriculation Number **LSC2009816** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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PROF. D. I. OLORUNFEMI **DATE**

(PROJECT SUPERVISOR)

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PROF.(MRS) E. T. AISIEN **DATE**

(HEAD OF DEPARTMENT)

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DECLARATION

I, **GIFT UGWUDIKE**, declare that "**GENOTOXIC EFFECTS OF VEHICULAR EMISSIONS ON THE DNA OF BUCCAL CELLS IN DRIVERS AT CENTRAL PARK, SAPELE ROAD, BENIN CITY: A MICRONUCLEI ASSAY -BASED STUDY**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other university.

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GIFT UGWUDIKE (MISS) **DATE**

DEDICATION

It is with a genuine heart filled with love that I dedicate this report in good faith to my parents,

Mr and Mrs UGWUDIKE for their untold support right from the time I was conceived till date.

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ACKNOWLEDGEMENTS

I wish to register my profound gratitude to God Almighty for His undeserved kindness, mercy, compassion and loving-kindness towards me.

I also wish to sincerely commend the loving effort of my project supervisor in the person of Prof Daniel Olorunfemi for his guidance, encouragement and patience throughout the period of my project and my project writing despite his busy schedule. I also wish to appreciate the HOD Professor (Mrs) E.T Aisien for her motherly kindness and for providing an enabling environment for my project, my course adviser Dr Frank for his support from day one, Dr Jeffery Ogbebor and all my lecturers.

Lastly, I want to also acknowledge My parent's Mr and Mrs UGWUDIKE, my family, and my project colleagues for their support during the course of my project.

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ABSTRACT

Genotoxicity refers to the ability of certain physical or chemical agents to cause damage to the genetic material DNA and RNA within cells, thereby affecting their structural and functional integrity. This study was designed to evaluate the genotoxic effects of vehicular emissions on intra state drivers at central park Benin city. The central objective was to determine whether sustained exposure to vehicular fumes induces measurable genotoxic damage in these individuals. A total of 100 buccal cell samples were collected from 25 commercial bus drivers, each contributing four samples (two from each cheek). The samples were analyzed using the Buccal Micronucleus Cytome Assay.The results obtained from the exposed group were compared with 36 buccal cell samples collected from nine individuals who served as negative controls, each also contributing four samples. Altogether, 3,400 cells were examined under a light microscope 2,500 from the exposed group and 900 from the control group, to identify and quantify nuclear aberrations such as micronuclei, binucleated, and anucleated cells.The mean frequencies of micronucleated, binucleated and anucleated cells in exposed drivers where significantly higher ($p < 0.05$) than those in the control. By systematically comparing these findings, this study provides critical insights into the potential of vehicular emissions to induce genetic instability and cellular damage among occupationally exposed individuals. The outcomes underscore the biological impact of prolonged exposure to traffic-related air pollutants and highlight the urgent need for strengthened environmental policies and public health measures aimed at minimizing genotoxic risks in urban populations.

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CHAPTER ONE

INTRODUCTION

1.1 Background of study

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Genotoxicity refers to the property of chemical agents that can damage the genetic material—DNA and RNA—within a cell, thereby compromising its integrity and function (Olorunfemi *et al.*, 2023). Such damage can have serious biological consequences. In embryonic cells, genotoxic damage may result in heritable abnormalities and congenital defects, while in somatic cells, it can lead to carcinogenesis through the disruption of genetic material (Olorunfemi *et al.*, 2024). These potential health hazards have led to the development of various *in vitro* and *in vivo* toxicological techniques aimed at evaluating the genotoxic potential of chemical substances (Olorunfemi *et al.*, 2024). Genotoxicity testing serves as a critical tool in the identification of substances capable of inducing genetic alterations, either directly or through indirect DNA damage (Laun & Homa, 2021). Commonly employed genotoxicity assays include the Ames test, the comet assay, and the micronucleus assay, among others (Olorunfemi *et al.*, 2024). The micronucleus assay, in particular, has gained wide acceptance for its reliability in detecting chromosomal damage. Micronuclei are extranuclear bodies that contain chromosomal fragments or whole chromosomes which fail to integrate into the daughter nuclei during mitosis. This is typically due to errors in chromosomal segregation or spindle fiber attachment, reflecting clastogenic or aneugenic events (Kadeh *et al.*, 2022). The presence of micronuclei is considered a biomarker of genotoxic exposure and is strongly associated with an increased risk of cancer (Kadeh *et al.*, 2022). Consequently, the micronucleus assay is extensively used for assessing exposure to mutagens, carcinogens, and other agents that may induce DNA damage. The oral epithelium is a self-renewing tissue, maintained through the continuous proliferation of basal

cells, which migrate towards the surface and replace exfoliated cells (Vassoler *et al.*, 2021). The detection of micronucleated cells in exfoliated oral epithelial tissue is a recognized indicator of chromosomal damage and cancer risk (Vassoler *et al.*, 2021). Because the oral mucosa is frequently exposed to environmental and occupational genotoxins through ingestion and inhalation, it serves as an effective site for early biomonitoring of genotoxic events (Baptista *et al.*, 2025). Furthermore, since approximately 90% of all cancers are of epithelial origin, the buccal mucosa represents a strategic tissue for the early detection of potentially carcinogenic genetic alterations (Baptista *et al.*, 2025). A study conducted by Pagad *et al.* (2020) revealed a significantly high incidence of genotoxic effects among motorized tricycle drivers. The primary source of exposure was identified as automobile exhaust, which contains numerous genotoxic agents. These findings underscore the occupational health risks associated with prolonged exposure to vehicular emissions and highlight the importance of genotoxicity screening in high-risk populations.

1.2 Justification of study

Vehicular emissions are a major source of air pollution in urban environments, particularly in rapidly developing cities such as Benin City, Nigeria. Intra-state commercial drivers are among the most exposed populations due to their prolonged daily interaction with traffic emissions, often in conditions lacking adequate environmental regulation or protective measures. Despite growing global concern over the genotoxic potential of pollutants such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, and particulate matter, there is a significant gap in localized data on the biological effects of these emissions in Nigeria. DNA damage caused by environmental pollutants is a critical early event in the development of various diseases, including cancer. The buccal micronucleus cytome assay (BMCA) is a non-invasive, reliable,

and cost-effective technique for detecting genotoxic effects in exposed individuals. It provides important insight into chromosomal damage, genome instability, and cytotoxicity, making it an ideal method for population-based biomonitoring. Currently, there is a paucity of data linking vehicular emission exposure to genotoxic outcomes in populations residing or working in high-traffic areas within Benin City. This study will serve to fill this knowledge gap by providing empirical evidence on the extent of DNA damage in intra-state drivers, who represent a high-risk occupational group. Findings from this research will not only enhance the understanding of environmental health risks associated with urban transportation systems in Nigeria but may also inform policy decisions, occupational health guidelines, and public health interventions aimed at reducing exposure and improving air quality. Additionally, it will contribute to the growing body of evidence necessary for the advocacy of stricter emission control standards and urban planning reforms.

1.3 Aim

To investigate the genotoxic effects of prolonged exposure to vehicular emissions on the DNA integrity of buccal epithelial cells in intra-state drivers in Benin City, using the micronuclei assay as a biomarker for DNA damage, with the aim of contributing to occupational health risk assessment and environmental safety policies.

1.4 Objectives

1.To evaluate the frequency of micronuclei in buccal epithelial cells of intra-state drivers exposed to vehicular emissions in Benin City.

2. To compare the micronuclei frequency between intra-state drivers and a control group with minimal exposure to vehicular emissions.

3. To assess the correlation between the duration of occupational exposure (years of driving) and the extent of DNA damage in buccal cells.

4. To establish buccal micronucleus cytome assay as a non-invasive biomarker for early detection of genotoxic damage due to vehicular pollution.

5. To contribute data towards public health policy regarding occupational exposure to air pollution in urban transportation settings.

6. To raise awareness about the potential health risks of long-term exposure to vehicular emissions among intra-state drivers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Genotoxicity: An Overview

Genotoxicity refers to the ability of certain physical or chemical agents to damage the genetic material—DNA and RNA—within a cell, thereby compromising its integrity (Olorunfemi *et al.*, 2024). This damage can lead to mutations, which may have significant biological consequences, including the development of cancers and hereditary diseases. Genotoxic agents, or mutagens, include environmental pollutants, radiation, and various chemical substances that can induce structural alterations in DNA or chromosome (Olorunfemi *et al.*, 2024). Genetic damage can occur in both somatic and germ cells. In somatic cells, such alterations may result in malignant transformation, leading to cancer. In germ cells, DNA damage can cause inherited abnormalities and congenital disorders. Consequently, assessing the genotoxic potential of chemicals is a critical aspect of toxicological research and public health safety.

Testing Strategies for Genotoxicity

Genotoxicity testing is essential for identifying substances with the potential to induce genetic damage. A combination of *in vitro* and *in vivo* testing methods is employed to evaluate a substance's potential to affect human health (Luan and Honmu , 2021).

In vitro Genotoxicity Tests

In vitro tests are conducted using bacterial systems, cultured mammalian cells, or genetically engineered cell lines. These assays are relatively simple, cost-effective, and provide rapid results. Common *in vitro* assays include: Bacterial reverse mutation assays (Ames Test), mammalian

chromosome abberation test, micronucleus test, mouse lymphoma assay (Luan and Honmu, 2021).

However, a limitation of *in vitro* tests is the lack of metabolic activity in the test cells, particularly the absence of drug-metabolizing enzymes such as cytochrome P450 (CYP). To address this, a metabolic activation system called S9 mix—a supernatant derived from centrifuged liver homogenates (often from rats pre-treated with enzyme inducers like phenobarbital and 5,6-benzoflavone)—is added. The S9 mix mimics *in vivo* metabolism and enables the detection of genotoxic metabolites (Luan and Honmu , 2021). Alternatively, genetically modified cells expressing human CYP enzymes or primary hepatocytes with residual metabolic activity may be used (Luan and Honmu , 2021).

In vivo **Genotoxicity Tests**

In vivo assays are conducted using whole organisms, typically rodents such as mice and rats, and are designed to reflect the complex biological processes of metabolism, absorption, distribution, and excretion. These tests provide more accurate data for human risk assessment than *in vitro* assays. Common target tissues include bone marrow and peripheral blood, though specific organs may be selected based on the substance's expected route of exposure (e.g., dermal exposure in cosmetic testing) (Luan and Honmu , 2021).

Integrated Testing Approach

No single test can detect all forms of genotoxicity. Therefore, international regulatory frameworks recommend using a battery of complementary assays to cover a broad range of endpoints. The Organisation for Economic Co-operation and Development (OECD) has

established over 60 toxicity testing guidelines, of which 13 pertain specifically to genotoxicity. These tests are classified under:

In vitro Genotoxicity Tests

In vivo Genotoxicity Tests

In 2012, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) introduced the S2(R1) guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. This guidance outlines a standard testing strategy comprising:

A bacterial reverse mutation assay (Ames test) – *in vitro*

A chromosome aberration test, micronucleus test, or mouse lymphoma assay using mammalian cells – *in vitro*

A micronucleus or chromosome aberration test targeting hematopoietic tissue in rodents – *in vivo*

This structured approach ensures comprehensive detection of genotoxic agents across various biological systems and endpoint.

2.2 Vehicular Emissions and Genotoxicity

The rapid pace of industrialization and urbanization in recent decades has led to a marked increase in vehicular traffic, making motor vehicle emissions a dominant source of ambient air pollution in urban environments worldwide. Among the many adverse consequences of this environmental burden, the genotoxic potential of vehicular emissions has gained increasing attention due to its implications for public health. Numerous epidemiological and toxicological studies have demonstrated a strong association between high levels of ambient air pollution and a broad spectrum of health problems, including respiratory and cardiovascular diseases,

reproductive dysfunction, various cancers, and increased morbidity and mortality (Lewtas, 2007; Yang and Omaye, 2009). These deleterious health outcomes are largely attributed to the complex physical and chemical properties of airborne particulate matter (PM), a key component of vehicular emissions (Valavanidis *et al.*, 2008). Particulate matter, particularly fine particles ($PM_{2.5}$, aerodynamic diameter <2.5 μm) and ultrafine particles (UFPs, <100 nm), poses a significant threat to human health due to their ability to carry and adsorb toxic substances on their surface and penetrate deeply into the respiratory tract. Once inhaled, these particles can translocate to various organs and tissues, initiating a cascade of adverse biological effects. One of the primary mechanisms through which PM exerts its toxicity is the generation of reactive oxygen species (ROS), either directly or indirectly through the activation of inflammatory pathways (Risom *et al.*, 2005). This oxidative stress can lead to damage to lipids, proteins and nucleic acids, ultimately resulting in genotoxicity and increased risk of mutagenesis and carcinogenesis. Over the past few decades, the composition of urban air pollution has undergone a notable transformation. While regulatory measures and technological advancements have led to reductions in emissions from traditional sources such as coal and other fossil fuels, vehicular exhaust emissions have emerged as a major contributor to airborne PM, particularly in densely populated and traffic-congested areas (Valavanidis *et al.*, 2008). The increase in traffic density has led to elevated emissions of fine and ultrafine PM, as well as a range of harmful organic compounds, including polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs), both of which are associated with genotoxic and carcinogenic effects. Polycyclic aromatic hydrocarbons, in particular, are well-established mutagens and carcinogens. They are lipophilic, allowing them to persist in the environment and bioaccumulate in human tissues. Upon entering the body, PAHs are metabolized by cytochrome P450 enzymes such as CYP1A1,

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leading to the formation of reactive intermediates such as quinones that are capable of inducing oxidative stress and directly damaging DNA (Xue and Warshawsky, 2005). Similarly, VOCs like benzene represent a serious genotoxic threat due to their capacity to form reactive metabolites that can bind covalently to macromolecules such as DNA and proteins, thus interfering with critical cellular processes (Snyder and Hedli, 1996; Barreto *et al.*, 2009). The complexity of the genotoxic risk posed by vehicular emissions is further compounded by various modulating factors. The composition of particulate matter and associated pollutants is not constant; it varies significantly depending on factors such as geographic location, traffic patterns, industrial activity, and seasonal weather conditions. For example, PM concentrations and chemical profiles may differ between summer and winter due to variations in fuel combustion efficiency and atmospheric dispersion. Moreover, individual susceptibility to the genotoxic effects of these pollutants is influenced by a host of genetic and lifestyle factors. Genetic polymorphisms affecting xenobiotic metabolism, DNA repair capacity, and antioxidant defenses can significantly modify an individual's vulnerability to environmental genotoxins. Other confounding variables such as age, gender, dietary habits, smoking status, occupational exposure, and overall health also play a critical role in determining the biological response to air pollution (Romieu *et al.*, 2008; Lewtas, 2007). In light of these complexities, biomonitoring studies have become essential tools for assessing the health risks associated with vehicular emissions. By measuring biomarkers of exposure (e.g, levels of PAHs or benzene metabolites in biological fluids), dose (e.g, DNA adducts, oxidative DNA damage), and susceptibility (e.g, genotyping of metabolic and DNA repair genes), researchers can more accurately evaluate the genotoxic burden on vulnerable populations. Particular attention is given to individuals with high occupational or environmental exposure, such as traffic policemen, bus and taxi drivers, street vendors, and residents of high-

traffic urban areas. These studies not only help to identify the most at-risk individuals but also provide crucial insights into the molecular mechanisms underlying pollution induced genotoxicity. The rapid and sustained growth in global vehicle numbers over recent decades has led to a substantial increase in engine emissions, which have become a major contributor to urban air pollution (Health Effects Institute, 2010). This rise in vehicular traffic has occurred alongside a growing body of scientific evidence underscoring the severe health consequences of prolonged exposure to traffic related air pollutants.

2.3 Chronic Exposure in Drivers

Research has highlighted associations between traffic related air pollution and poor birth outcomes (Smith *et al.*, 2017), suboptimal lung development in children (Mudway *et al.*, 2019), and impaired cognitive development (Pedrerol *et al.*, 2017). Furthermore, there is strong epidemiological evidence connecting long-term exposure to these pollutants with the onset and exacerbation of chronic respiratory conditions (Gehring *et al.*, 2015; Pfeffer *et al.*, 2018; Samoli *et al.*, 2016), as well as with the development of cardiovascular diseases (Alexeeff *et al.*, 2018; Atkinson *et al.*, 2010; Bell *et al.*, 2014). Additional studies have found increased risks of dementia (Carey *et al.*, 2016), various forms of cancer (Hart *et al.*, 2015), and ultimately, premature mortality (Atkinson *et al.*, 2016; Hoek *et al.*, 2002) attributable to long-term exposure to vehicular emissions. In parallel to health concerns, the environmental implications of vehicle emissions have also come into focus. The marked increase in vehicle use, particularly diesel vehicles, has heightened concerns regarding their contribution to global warming. In the 1990s, in an attempt to curb carbon dioxide (CO_2) emissions, the European Commission introduced incentives favoring diesel vehicles over gasoline powered alternatives due to their relatively lower CO_2 output (Cames and Helmers, 2013). As a result, Europe witnessed a dramatic surge in

diesel vehicle registrations, with an increase of approximately 45 million diesel cars over two decades. Diesel vehicles grew from 27.1% of the total European vehicle fleet in 2005 to 42.4% by 2017 (European Environment Agency, 2018). Despite their lower CO_2 emissions, diesel vehicles have raised serious concerns due to their disproportionately high output of air pollutants such as fine particulate matter (PM) and nitrogen oxides (NO_x). Regulatory findings indicate that diesel engines emit significantly more fine particulate matter than gasoline engines, with estimates suggesting that diesel vehicles contribute to over 90% of total vehicular exhaust emissions in the United Kingdom (Monks *et al.*, 2012). This disproportionate contribution to pollution has serious public health ramifications, as exposure to diesel exhaust has been classified as carcinogenic to humans (Group 1 carcinogen) by the International Agency for Research on Cancer (IARC). Initial evidence of the health impacts of traffic-related air pollution emerged from epidemiological studies comparing the health of populations residing near high-traffic roads. These studies consistently reported elevated rates of cardiopulmonary mortality, asthma, and diminished lung function in populations living adjacent to major roadways (Brunekreef *et al.*, 2009; Health Effects Institute, 2010; Hoek *et al.*, 2002; Janssen *et al.*, 2003). One pivotal study found that increased diesel truck traffic directly correlated with a worsening of asthma symptoms in nearby residents (Brunekreef *et al.*, 2009). In recent years, methodological advances have allowed researchers to refine exposure assessments by linking health data to individual level estimates of pollution exposure. These studies often use nitrogen dioxide (NO_2) as a proxy for traffic-related air pollution due to its strong association with vehicle emissions (Atkinson *et al.*, 2016; Samoli *et al.*, 2016). Within this context, particular attention has been drawn to the urban commuting microenvironment a setting where individuals may experience disproportionately high pollution exposures. Although the average person spends only 6–10% of

their day commuting, studies have shown that this relatively short period can account for 20–30% of their total daily exposure to air pollutants (Dons *et al.*, 2012; Williams and Knibbs, 2016). Moreover, the concentration of pollutants in vehicles or on roads during peak traffic periods can be up to eight times higher than in the home environment (Dons *et al.*, 2011). For professional drivers such as taxi drivers, delivery personnel, bus operators, and truck drivers, the risks are even more pronounced. Their prolonged daily exposure within confined vehicular spaces, often with limited ventilation and in high-traffic areas, places them at increased risk of chronic exposure to harmful airborne pollutants. Studies have consistently indicated elevated exposure levels in these occupational groups, with long term health consequences that mirror, and sometimes exceed, those observed in general populations. Given the classification of diesel exhaust as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), the implications for individuals with long-term occupational exposure such as professional drivers are particularly alarming. The evidence suggests that chronic exposure to high levels of traffic related pollutants, especially in urban commuting environments, constitutes a serious and often under recognized public health issue. As such, reducing emissions, improving in-vehicle air quality, and implementing regulatory strategies targeting urban traffic density are crucial public health interventions that warrant urgent attention.

2.4 Buccal Cells as Bioindicator

The buccal mucosa, a stratified squamous epithelium composed of four distinct cellular layers, represents a highly accessible and minimally invasive tissue for sampling in both clinical and research contexts. Due to its anatomical location and structural properties, the buccal epithelium has been increasingly recognized as an effective bioindicator for assessing cellular health, genomic stability, and environmental or occupational exposure to genotoxic agents (Mladinic *et*

al., 2022). This tissue not only allows for the non-invasive collection of cells but also provides a unique window into systemic and localized biological responses to various internal and external stimuli (Knasmueller *et al.*, 2024).

Histologically, the buccal mucosa consists of multiple layers of cells that maintain their integrity through continuous cell renewal. Basal cells, located at the deepest layer of the epithelium, undergo mitotic division, giving rise to daughter cells that migrate upward through the layers, differentiate into squamous epithelial cells, and are eventually exfoliated into the buccal cavity (Mladinic *et al.*, 2022). These exfoliated cells can be easily collected using a simple swab technique, offering a practical and ethical advantage for repeated sampling in longitudinal studies and large scale population screenings. Functionally, the buccal mucosa serves as a primary biological barrier to agents entering the body through inhalation or ingestion. It is significantly more permeable by a factor of approximately 4 to 4000 than the skin (Knasmueller *et al.*, 2024). This is attributed to its rich vascular and lymphatic supply, and its ability to bypass first pass hepatic metabolism and gastrointestinal degradation. These characteristics make the buccal mucosa not only a susceptible site for exposure to genotoxic and carcinogenic substances, but also a valuable target for evaluating the biological effects of such exposures. The buccal micronucleus cytome assay (BMCyt assay) has emerged as a powerful biomonitoring tool in this context. Introduced in 1982 to assess genotoxic effects from betel quid chewing, the buccal micronucleus (MN) assay has since evolved and found widespread application in occupational, environmental, nutritional, pharmaceutical, and clinical research (Knasmueller *et al.*, 2024). The assay is designed to detect and quantify various biomarkers of genetic damage, including micronuclei (MN), binucleated cells, and other nuclear anomalies. These biomarkers reflect chromosomal breakage or missegregation events that occur during cell division, particularly in

the basal layer of the mucosa, where proliferating cells are most vulnerable to DNA damage (Mladinic *et al.*, 2022). Genotoxic events affecting basal cells can lead to the formation of micronucleus (MN)-small, extranuclear bodies that arise from acentric chromosomal fragments or whole chromosomes that fail to incorporate into the daughter nuclei during mitosis (Knasmueller *et al.*, 2024). These damaged cells then undergo differentiation and are eventually exfoliated, allowing for the assessment of cumulative genotoxic damage through cytological examination. Recent advancements in the methodology have led to the development of the buccal MN cytome (BMCyt) assay, an enhanced version of the original technique. This upgraded assay incorporates the scoring of a wider array of cell types and nuclear anomalies, enabling a more comprehensive assessment of genome instability, cell death (including apoptosis and necrosis), and cytokinetic defects (Mladinic *et al.*, 2022). The cytome approach not only detects DNA damage but also elucidates cellular mechanisms of toxicity and disease progression. The relevance and reliability of the buccal MN assay as a bioindicator have been validated in numerous studies over the past four decades (Knasmueller *et al.*, 2024). It has been successfully employed in biomonitoring populations exposed to air pollutants, heavy metals, pesticides, and industrial chemicals. Furthermore, it has proven valuable in clinical settings, such as monitoring patients undergoing chemotherapy or radiotherapy, and in evaluating chromosomal instability in individuals with various cancers, precancerous conditions, and chronic diseases such as diabetes, neurodegenerative disorders, and autoimmune diseases (Mladinic *et al.*, 2022). Moreover, the buccal MN assay has gained attention as a tool to investigate the impact of lifestyle and nutritional factors on genomic integrity. Diet, alcohol consumption, tobacco use, and other behavioral variables have been shown to influence the frequency of MN and other nuclear abnormalities in buccal cells, highlighting the assay's utility in preventive and personalized

medicine. Claudia Bolognesi *et al.*, (2014) and other leading researchers have emphasized that the buccal mucosa, due to its anatomical and functional characteristics, is a high risk site for genotoxic insult from agents entering via the aerodigestive tract (Mladinic *et al.*, 2022). The high cell turnover in the oral epithelium makes it particularly suitable for capturing and reflecting transient as well as chronic genotoxic exposures . Overall, the use of buccal cells as a bioindicator offers numerous advantages, including its non-invasive nature, ease of sample collection, and the ability to detect early biomarkers of genetic and cellular damage (Knasmueller *et al.*, 2024). The buccal MN cytome assay stands as a robust, sensitive, and cost effective tool for biomonitoring human exposure to genotoxic agents and assessing individual and population level genome stability (Mladinic *et al.*, 2022). Its growing applications in environmental health, clinical diagnostics, epidemiology, and toxicology underscore its significance as a cornerstone in modern biomonitoring and public health research (Knasmueller *et al.*, 2024).

2.5 Micronucleus Assay: A Genotoxicity Measurement

The micronucleus (MN) assay is a widely recognized and extensively utilized cytogenetic technique for the detection of genotoxic events and chromosomal instability (kaddah *et al.*, 2022). It is employed in both *in vitro* and *in vivo* settings to assess DNA damage caused by exposure to mutagens, carcinogens, and clastogens, making it an essential tool in genetic toxicology, environmental monitoring, and cancer risk assessment (laun and Homa, 2021). Micronuclei are small extranuclear bodies that form during cell division when chromosome fragments or entire chromosomes fail to be incorporated into the daughter nuclei. This occurs due to structural or numerical chromosomal aberrations, commonly referred to as clastogenic and

aneugenic events, respectively. Clastogenic effects lead to chromosomal fragments due to DNA breaks, while aneugenic effects result in whole chromosomes being excluded from the main nucleus due to mitotic spindle defects or missegregation (kaddah *et al.*, 2022). During mitosis, especially at anaphase, all chromosomes are expected to migrate to opposite poles of the dividing cell, guided by the mitotic spindle apparatus. However, when there is a disruption in this mechanism, chromosome fragments or entire chromosomes may lag behind and remain in the cytoplasm (laun and Honma, 2021). These acentric fragments or whole chromosomes are subsequently encapsulated by a nuclear membrane, forming what is known as a micronucleus. These micronuclei stain similarly to the main nucleus and contain either a chromosomal fragment or a whole chromosome that was not incorporated into one of the daughter nuclei. According to several studies, including those by Bonassi *et al.*, (2017) and other researchers, micronuclei are considered reliable biomarkers of genomic instability, DNA damage, and increased risk of various diseases, including cancer and cardiovascular disorders. The frequency of micronuclei in cells, especially in peripheral blood lymphocytes and exfoliated epithelial cells correlates with both past and present exposure to genotoxic agents, making the MN assay an effective biomonitoring tool (kaddah *et al.*, 2022).The International Human Micronucleus Project (HUMN) has validated the micronucleus test, particularly in human lymphocytes and buccal mucosa cells, as a robust and predictive assay for assessing genotoxic exposure and cancer risk in population studies. Its increasing utility in clinical, occupational, and environmental settings highlights its importance in public health surveillance. Types of cells used in micronucleus Assay includes

1. Peripheral blood lymphocytes: Frequently used due to ease of collection and well-established protocols.

2. Exfoliated epithelial cells: Especially from the buccal mucosa, offer a non-invasive method for evaluating genotoxicity in target tissues of inhaled or ingested toxicants.

3. Cultured mammalian cells: Including Chinese hamster ovary (CHO) cells, V79 cells, and human lymphoblastoid lines, are used for standardized *in vitro* assays.

4. Bone marrow cells: Used predominantly in *in vivo* rodent studie (kaddah *et al.*, 2022).

The Micronucleus Assay procedures includes:

In vitro Micronucleus (MN) Test:

In vitro micronucleus testing involves exposing cultured cells to a test compound and subsequently preparing slides to observe micronuclei in interphase cells (kaddah *et al.*, 2022). The assay determines the ability of the substance to induce chromosomal breakage or mitotic spindle interference, thus resulting in the formation of micronuclei. One enhancement to this procedure is the use of Cytochalasin B (Cyto B), a cytokinesis-blocking agent that inhibits actin polymerization. This allows cells to undergo nuclear division without completing cytokinesis, resulting in binucleated cells. Scoring is then limited to these binucleated cells, ensuring that only dividing cells are evaluated, which improves the accuracy and sensitivity of the assay. However, Cyto B is not used in rapidly proliferating cell lines due to their high mitotic index, which allows easy identification of dividing cells even without cytokinesis blockade (laun and Honma , 2021).

In vivo Micronucleus (MN) Test:

In vivo assays typically involve administering the test substance to rodents and collecting bone marrow or peripheral blood samples. The frequency of micronucleated erythrocytes particularly immature polychromatic erythrocytes (PCEs) is then evaluated. This assay provides an

assessment of systemic genotoxicity and is often part of regulatory toxicology packages (kaddah *et al* ., 2022)

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The Fluorescences in situ hybridization (FISH) based centromere staining to distinguish between structural and numerical chromosomal aberrations, the micronucleus assay can be complemented with fluorescence in situ hybridization (FISH) (Kaddah *et al.*, 2022). This technique employs centromere specific DNA probes to identify the presence of centromeres in micronuclei. Centromere positive (CEN+) micronuclei indicate the presence of whole chromosomes, suggesting aneugenic effects. Centromere negative (CEN–) micronuclei indicate acentric fragments, pointing to clastogenic effects.This differentiation is crucial for mechanistic understanding and regulatory interpretation of genotoxicity data, especially in the assessment of pharmaceutical agents, pesticides, and industrial chemicals.

The micronucleus test is endorsed by multiple regulatory agencies including: Organisation for Economic Co-operation and Development (OECD) (Test Guidelines 474 for *in vivo* and 487 for *in vitro* assays), International Conference on Harmonization (ICH)(for pharmaceutical genotoxicity testing), Environmental Protection Agency (EPA), European Chemicals Agency (ECHA), and Food and Drug Administration (FDA) for environmental and chemical safety evaluations.It is also increasingly used in cancer epidemiology, occupational health monitoring, nutritional and lifestyle intervention.

Advantages of the Micronucleus Assay:

Simplicity and Efficiency.

Does not require metaphase arrest or karyotyping, making it less labor-intensive than traditional chromosomal aberration tests.

Broad Applicability: Can detect both clastogenic and aneugenic events.

Relevance to Human Health: Correlates with disease risk and environmental exposure.

Versatility Applicable *in vitro*, *in vivo*, and in human biomonitoring.

High Throughput Easily automated for large-scale screening. (Iaun and Honma, 2021)

Limitations and Considerations.

While the micronucleus assay is a powerful tool, certain limitations should be acknowledged.

Requires clear identification of dividing cells, especially in *in vitro* settings.

False positives may occur due to cytotoxicity or apoptosis.

In vitro Interpretation may be complicated in tissues with naturally high rates of binucleated cells. (Kaddah *et el*., 2022).

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Standardization in scoring criteria is essential to ensure reproducibility across laboratories. The micronucleus assay stands as a cornerstone in genotoxicity testing, offering a reliable, efficient, and mechanistically informative method for assessing chromosomal damage. Its role in environmental monitoring, human biomonitoring, and chemical safety evaluation continues to expand, driven by advances in cytogenetics and molecular biology. The integration of complementary techniques like Fluorescence In Situ Hybridization (FISH) further enhances its utility, enabling researchers and regulatory bodies to distinguish between different types of genotoxic insults with high specificity (Laun and Honma , 2021).

2.6 Impact of Vehicular Emissions on Oxidative Stress and DNA Damage

Environmental air pollution has become one of the most critical global public health concerns of the 21st century (Raj *et al.*, 2021). Numerous epidemiological studies have consistently demonstrated a strong correlation between air pollution and increased morbidity and mortality, particularly in urban and industrialized regions (Raj *et al.*, 2021). A major contributor to this burden is vehicular emissions, which constitute a complex mixture of harmful gases and

particulate matter (PM) capable of triggering a cascade of adverse health outcomes via inflammation and oxidative stress mechanisms. Ambient air pollution is composed of a diverse and complex mixture of toxicants including particulate matter (PM), volatile organic compounds (VOCs), irritant gases (e.g., ozone, nitrogen oxides, sulfur dioxide), and known carcinogens such as benzene and polycyclic aromatic hydrocarbons (PAHs). Particulate matter, a principal component of polluted air, is broadly classified by aerodynamic diameter into PM10 (particles ≤10 μm), PM2.5 (particles ≤2.5 μm), and ultrafine particles (UFPs, particles <100 nm). The chemical composition of PM varies significantly depending on geographical location, meteorological conditions, and the nature of emission sources (ladovici *et al.*, 2021). Common constituents include inorganic ions (sulfates, nitrates, ammonium, and chlorides), trace metals, elemental and organic carbon, biological components (bacteria, spores, pollens), and adsorbed volatile and semi volatile organic compounds.Vehicular emissions especially from diesel engines are a dominant source of urban PM, particularly ultrafine particles (Rossne *et al.*, 2008). Diesel exhaust particles (DEPs) consist of a carbonaceous core coated with adsorbed organic compounds, metals, and other pollutants (Raji *et al.*, 2021). These fine and ultrafine particles have large surface areas relative to their mass, enhancing their capacity to carry and deliver toxic substances into the respiratory system. Once inhaled, ultra fine particles (UFPs) exhibit a high alveolar deposition rate due to their small size and can translocate into the systemic circulation, triggering systemic inflammation and oxidative stress in extrapulmonary organs. Oxidative stress arises when there is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the body. ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH), are produced through both endogenous metabolic processes and exogenous environmental exposures such as air pollution (ladovici *et al.*,

2011) . In the context of vehicular emissions, ROS generation is facilitated by redox active components of Particulate Matter (PM), such as transition metals (e.g., iron, copper, vanadium), polycyclic aromatic hydrocarbons (PAHs), and volatile organic carbons (VOCs). PAHs found in traffic related PM are metabolized by cytochrome P450 enzymes (notably CYP1A1) into reactive intermediates such as quinones (Rosser *et al.*, 2008) . These metabolites contribute to oxidative stress both directly through redox cycling and indirectly through the formation of electrophilic species that bind covalently to DNA and proteins (Raji *et al.*, 2021). Transition metals adsorbed on the surface of PM can catalyze Fenton type reactions, leading to the formation of hydroxyl radicals, the most reactive and damaging form of ROS (Lodovici *et al.*, 2011). Additionally, nitric oxide (NO) and its derivatives collectively known as reactive nitrogen species (RNS) plays a dual role. While NO is essential for vascular homeostasis and immune modulation, excessive NO production, especially in the presence of superoxide, results in peroxynitrite ($ONOO^-$) formation, a potent oxidant that exacerbates tissue damage and impairs cellular functions.The health effects of PM are not limited to the respiratory system (Lai *et al.*, 2005) . Upon inhalation, particles deposited in the alveoli activate resident macrophages and epithelial cells, resulting in the release of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and chemokines. This localized inflammation can extend systemically (Raj *et al.*, 2021), especially when ultrafine particles or their soluble components translocate into the bloodstream. Systemic oxidative stress and inflammation are now recognized as central mechanisms linking air pollution exposure to a wide range of diseases, including cardiovascular and cerebrovascular diseases, diabetes mellitus, neurodegenerative disorders such as Alzheimer's disease, and various forms of cancer (Raj *et al.*, 2021). Chronic inflammation triggered by continuous exposure to traffic related PM may promote endothelial dysfunction, arterial stiffening, atherosclerotic

plaque formation, and even thrombogenesis. One of the hallmark outcomes of oxidative stress is damage to DNA. ROS can attack nucleic acids, particularly guanine bases, resulting in the formation of mutagenic lesions. Among these, 8-hydroxy-2'-deoxyguanosine (8-OHdG or 8-oxodG) is the most commonly studied biomarker of oxidative DNA damage (Lai *et al.*, 2005). The hydroxyl radical reacts with guanine to form 8-oxodG, which, if unrepaired, can cause GC→TA transversions, a type of mutation linked to carcinogenesis (Lai *et al.*, 2005) However, when repair mechanisms are active, 8-oxodG is excised from DNA and excreted in urine, where it can be quantified by sensitive analytical methods such as liquid chromatography-mass spectrometry (LC-MS). Elevated urinary levels of 8-OHdG have been consistently associated with high exposure to traffic-related air pollution. Another widely used biomarker is 1-hydroxypyrene (1-OHP), a metabolite of pyren, a representative PAH. Urinary levels of 1-OHP or its glucuronide conjugate (1-OHPG) have been employed as exposure indicators for PAH rich vehicular emissions. Studies indicate a dose response relationship between traffic exposure and urinary 1-OHPG, underscoring its potential as a reliable biomarker for assessing recent PAH exposure. Long term exposure to traffic related air pollution is strongly associated with the development of chronic diseases such as lung cancer, chronic obstructive pulmonary disease (COPD), ischemic heart disease, and stroke. Prolonged oxidative stress and chronic low grade inflammation act synergistically to initiate and promote these conditions. In children, early life exposure may impair lung development and increase the risk of asthma and allergic sensitization. In adults, it can accelerate biological aging and exacerbate pre-existing health conditions (Rossner et el., 2008). Short-term exposure peaks often observed during traffic congestion or pollution episodes can also have immediate effects, such as acute asthma exacerbations,

bronchitis, reduced heart rate variability, increased blood pressure, and even triggering of myocardial infarctions in susceptible individuals (lai *et al.*, 2005).

2.7 DNA repair mechanism

DNA is the fundamental blueprint of life, and its integrity is critical for normal cellular function, organismal development, and health (Olorunfemi *et al.*, 2024). However, DNA is continually exposed to damaging agents, both endogenous (e.g., reactive oxygen species, replication errors) and exogenous (e.g., ionizing radiation, chemicals, and pollutants) (Olorunfemi *et al.*, 2024). Among the most significant environmental genotoxins are those found in vehicular emissions, including polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), nitrogen oxides (NOx), and heavy metals (laun and Honma, 2021). These agents pose particular risk to individuals with chronic exposure, such as traffic police, drivers, and urban residents. To mitigate damage caused by such exposure, cells have evolved a variety of DNA repair mechanisms that detect and correct genetic errors before they lead to mutations, carcinogenesis, or cell death (Olorunfemi *et al.*, 2024).

2.8 Mechanism of Genotoxicity

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The underlying mechanism of genotoxicity involves a complex interplay between reactive compounds and the cellular genetic machinery, particularly deoxyribonucleic acid (DNA) (Mohammed *et al.*, 2017) Such interactions can result in structural alterations to the DNA molecule, interference with its replication and repair processes, and modification of the chromosomal architecture, thereby compromising genomic integrity (Mohammed *et al.*, 2017).

Interaction of Genotoxic Substances with DNA

At the molecular level, genotoxic substances exert their harmful effects through direct or indirect interactions with the DNA structure and sequence. Direct interaction occurs when these agents form covalent or non-covalent bonds with the DNA molecule, leading to the formation of adducts, cross-links, or strand breaks. In contrast, indirect interaction involves the generation of reactive intermediates such as reactive oxygen species (ROS), electrophiles, or lipid peroxidation products that subsequently attack DNA and other macromolecules (Mohammed *et al.*, 2017). These interactions typically occur at specific locations or base sequences within the DNA strand (Mohammed *et al.*, 2017). The result of such interactions may include the formation of DNA lesions, single- or double-strand breaks, chromosomal fusion, base pair deletion, missegregation of chromosomes, or nondisjunction during mitotic and meiotic cell division. Collectively, these events can induce point mutations, structural chromosomal abnormalities, or aneuploidy, all of which are indicative of genotoxic stress (Mohammed *et al.*, 2017). For instance, when a genotoxic agent binds to a specific base such as guanine or adenine, it can alter the base-pairing properties, leading to mismatched replication during cell division. Over time, if the damage is not adequately repaired, these alterations may become permanent mutations that disrupt gene expression or activate oncogenes, promoting cancer development.

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Transition Metals and DNA Damage

Among the numerous genotoxic substances, transition metals such as chromium, nickel, and cadmium play a significant role in DNA damage due to their redox active properties. Chromium, particularly in its high valent oxidation state (Cr^{6+}), is one of the most potent metal based genotoxins. When chromium compounds enter the cell, they undergo a series of intracellular reduction reactions, generating intermediates such as Cr^{5+} and Cr^{4+}. These reactive intermediates

can interact directly with DNA, leading to the formation of chromium-DNA adducts, strand breaks, and oxidative lesions. Researchers have reported that these interactions result in various forms of DNA damage, including base oxidation, DNA-protein cross-linking, and the inhibition of DNA repair enzymes (Mohammed *et al.*, 2017). These molecular alterations can subsequently initiate carcinogenic processes (Mohammed *et al.*, 2017) . The mechanism of DNA damage caused by high valent chromium involves the generation of oxidative stress, which leads to the formation of DNA lesions relevant to in-vivo genotoxic effects observed in chromate exposed human populations. Prolonged exposure to hexavalent chromium compounds has been linked to lung cancer, nasal septum ulceration, and other malignancies among occupationally exposed workers. Consequently, high-valent chromium is classified as a confirmed human carcinogen by various international health agencies, including the International Agency for Research on Cancer (IARC) (Mohammed *et al.*, 2017).

Role of Reactive Oxygen Species (ROS)

Reactive oxygen species are among the most significant contributors to genotoxicity. These highly reactive molecules, which include hydroxyl radicals (•OH), superoxide anions ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2), are generated as natural by-products of normal cellular metabolism. Under physiological conditions, ROS play beneficial roles in cell signaling and homeostasis. However, excessive accumulation of these species, often due to environmental pollutants, radiation exposure, or xenobiotic metabolism, overwhelms the cellular antioxidant defense system, resulting in oxidative stress. One of the most prevalent oxidative lesions produced by ROS is 8-hydroxy-2'-deoxyguanosine (8-OHdG), which forms when guanine bases in DNA are oxidized. This lesion is particularly mutagenic, as it can mispair with adenine during replication, leading to G:C → T:A transversion mutations (Mohammed *et al.*, 2017) . Such mutations, if left

un-repaired, can disrupt the function of tumor suppressor genes or activate proto-oncogenes, fostering malignant transformation. Elevated levels of 8-OHdG in tissues and biological fluids are widely recognized as biomarkers of oxidative DNA damage and have been associated with numerous pathological conditions, including neurodegenerative disorders, cardiovascular diseases, and various types of cancer. ROS can also damage other cellular components such as lipids and proteins. Lipid peroxidation, for example, leads to the formation of reactive aldehydes like malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), both of which can form adducts with DNA and proteins, further exacerbating genotoxic and cytotoxic effects (Mohammed *et al.*, 2017).

Lipid Peroxidation and Secondary Reactive Products

Lipid peroxidation is a chain reaction process initiated by free radicals attacking polyunsaturated fatty acids in cellular membranes. The breakdown of lipid peroxides gives rise to secondary reactive aldehydes, including 4-hydroxynonenal (4-HNE), acrolein, and crotonaldehyde. Among these, 4-hydroxynonenal (4-HNE) has received particular attention due to its ability to covalently bind to nucleophilic sites in DNA and proteins, forming stable adducts that interfere with normal cellular function.

4-HNE can modify DNA bases such as deoxyguanosine, deoxyadenosine, and deoxycytidine, leading to the formation of exocyclic etheno-DNA adducts. These adducts are promutagenic and can induce point mutations, insertions, or deletions in critical genes. In addition to its direct genotoxic potential, 4-HNE influences numerous intracellular signaling pathways that regulate cell proliferation, apoptosis, and differentiation. It can modulate transcription factors such as nuclear factor erythroid 2–related factor 2 (Nrf2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby altering the expression of genes involved in oxidative

stress response and inflammation. Moreover, 4-HNE has been implicated in the pathogenesis of various oxidative stress-related diseases, including atherosclerosis, hepatic fibrosis, diabetes mellitus, and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Its ability to promote both cytotoxic and cytoprotective responses underscores its dual role in cellular physiology, acting as a toxic product at high concentrations while serving as a signaling molecule at low, sub-toxic levels.

2.9 Cellular Responses and DNA Repair Mechanisms

Cells have evolved multiple defense mechanisms to counteract genotoxic insults and preserve genomic stability. These include antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which neutralize ROS; and an array of DNA repair pathways that correct different types of DNA damage. Examples include base excision repair (BER) for small base modifications, nucleotide excision repair (NER) for bulky adducts, mismatch repair (MMR) for replication errors, and homologous recombination (HR) or non-homologous end joining (NHEJ) for double-strand breaks (Mohammed *et al.*, 2017). However, when the extent of damage exceeds the repair capacity of the cell, mutations accumulate, and apoptosis or uncontrolled proliferation may ensue. In the latter case, persistent DNA damage can initiate carcinogenesis by promoting genetic instability, leading to tumor initiation and progression (Mohammed *et al.*, 2017) .

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area:

The study was conducted in Central Park, Benin City, in the year 2025. A total of twenty-five (25) participants were selected from the study population, all of whom were commercial bus drivers. Prior to sample collection, informed consent was obtained from each participant. All procedures and data handling were carried out in strict compliance with established ethical principles and confidentiality protocols.

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3.2 Materials Needed

Wooden spatula

Physiological saline

Methylated spirit

Crayons reagent

Slide

Slide box

May Grunwald stain

Giemsa

Dropper

Coupling jar

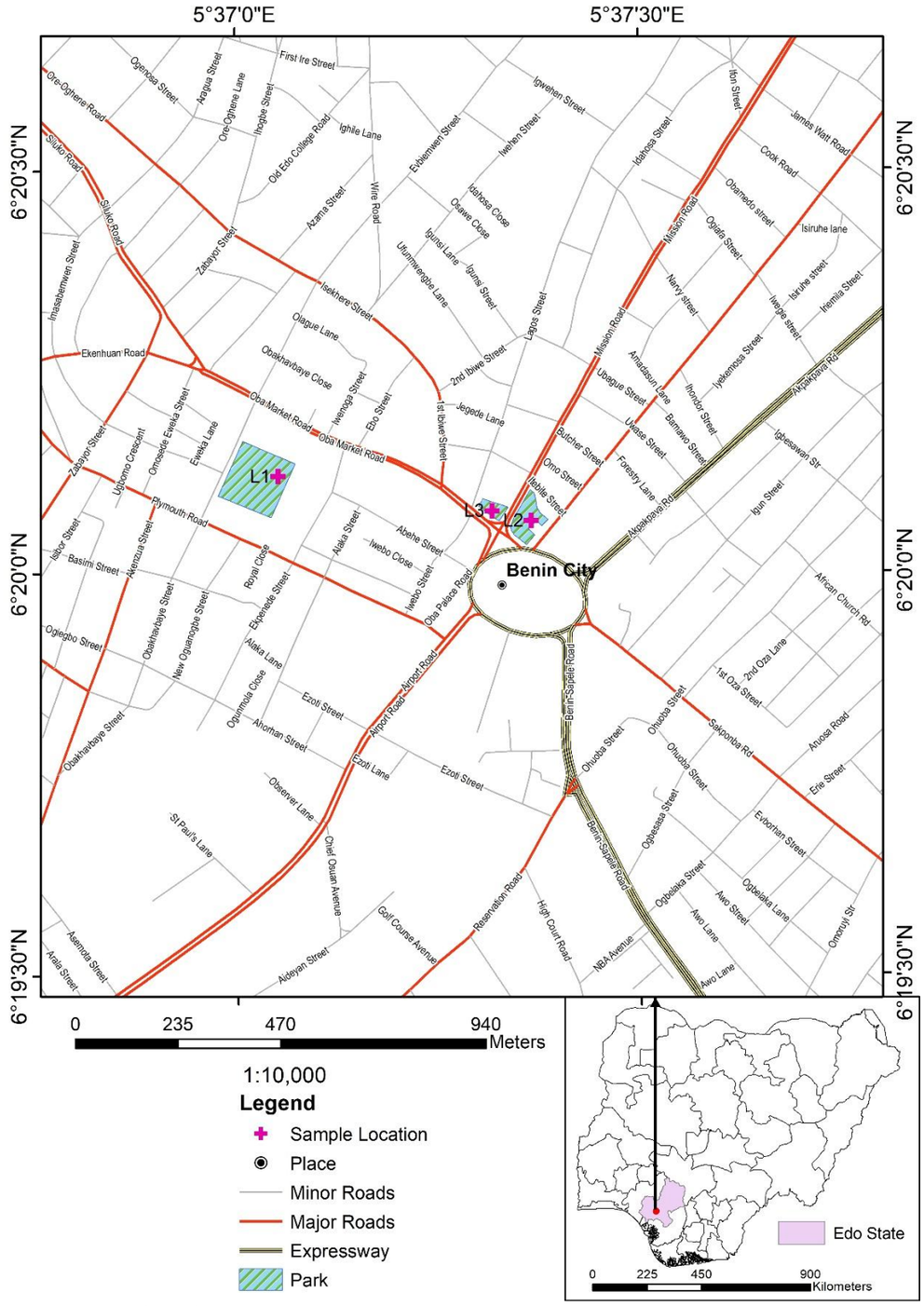

Fig 3.1 Map of study area

3.3 Sample Collection:

Four Oral mucosa samples were collected from each of the 25 participants (Having a total of 100 samples) two from each check following standard procedures. Each individual was first instructed to rinse their mouth thoroughly with clean water. A sterile spatula coated with saline was then provided and used to gently scrape the inner surfaces of both the right and left buccal (cheek) regions to obtain oral mucosal cells. The collected samples were immediately smeared onto pre-coded microscope slides that had been disinfected with methylated spirit and in which a drop of saline has been added using a dropper. It was then allow to dry for about 30 minutes. The prepared slides were then securely placed in a labeled slide box and transported to the laboratory for further analysis

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3.4 Fixation and Staining Procedure for Oral Mucosa Smear Preparation:

The coded slide was placed in Carnoy's fixative for 5 minutes. The fixative, consist of methanol and glacial acetic acid in a ratio of 3:1. All fixation procedures were conducted in a well-ventilated laboratory After fixation, the slide was air-dried for 24 hours and stained with May–Grünwald stain for 5 minutes, followed by gentle rinsing with distilled water. Subsequently, Giemsa stain was applied to the slide for 15 minutes to enhance nuclear visualization, cytoplasmic contrast and finally rinsed with distilled water and allowed to dry for 48 hours. This procedure was repeated for each of the collected samples.

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3.5 Analysis of Micronuclei (MN) and Other Nuclear Abnormalities:

The analysis of micronuclei (MN) and other nuclear abnormalities including micro nucleated cells (MN), binucleated cells (BNs), and additional morphological indicators of genotoxic and cytotoxic damage was meticulously conducted on pre-coded slides. Each sample was

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independently evaluated to ensure objectivity and consistency in data interpretation. 25 cells per slide was examined making a total of 100 cells per individual were systematically examined under a 100× oil immersion objective lens using a high-resolution light microscope. Each cell was carefully assessed for the presence of nuclear anomalies in accordance with internationally recognized cytogenetic criteria and established guidelines for nuclear morphology. These criteria encompassed evaluation of nuclear size, shape, staining pattern and intensity, chromatin distribution, and the alignment of nuclear structures within the same focal plane. Micronuclei were identified as small, round or oval chromatin-containing bodies located within the cytoplasm but distinctly separated from the primary nucleus, exhibiting the same staining characteristics and focal plane as the main nucleus. Other abnormalities, including binucleated cells, were classified following standardized definitions to ensure accurate differentiation between genuine nuclear alterations and artefacts. To validate the reliability and significance of the observed findings, negative control slides were prepared and analyzed alongside the test samples. The negative control provided a baseline reference for normal cellular morphology. The inclusion of these controls enabled a meaningful comparison, ensuring that the results obtained from the test samples could be interpreted with confidence. This rigorous analytical approach was designed to ensure reproducibility, minimize observer bias, and provide a reliable quantitative and qualitative estimate of nuclear damage within the evaluated cell populations.

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CHAPTER FOUR

RESULTS

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4.1 Demographic Characteristics of the Exposed and Control Group

Table 4.1 presents the demographic characteristics of both the exposed and control groups within the study. The gender distribution indicates that all participants in both groups (I,e both exposed and control) are male this is because the exposed group consist predominantly of professional drivers which is a male dominated occupation. The age distribution for the exposed group ranged from 20-50 years categorized into three age brackets 20 – 30 , 31-40 , and 41-50 years among the exposed individuals , 8 participant fell within the 20-30 age range , 14 participant within 31-40 and 3 participant within 41-50 years with corresponding mean ages of 25.6, 35.1 and 45.5 years respectively .Similarly, for the control group the age distribution followed the same pattern with 6 participant in the 20-30 age group 2 participant in the 31- 40 age group and 1 participant in the 41-50 age group the respective mean age for these groups were 24.23 , 32.20 and 41.0 years in addition to age the table also provides information on the educational status of both groups using the questionnaire presented in Appendix A. Individuals with a history of smoking were excluded to ensure that vehicular emissions remained the sole exposure variable under consideration. The questionnaire was also utilize to eliminate individuals with allergies or health conditions that could affect the results. This demographic break down provides a comprehensive understanding of the composition of the study population and ensures comparability between the exposed and control groups.

TABLE 4.1: Demographic characteristics of exposed and control groups.

EXPOSED			CONTROL		
GENDER	**NUMBER**	**PERCENTAGE**	**GENDER**	**NUMBER**	**PERCENTAGE**
MALE	25	100%	MALE	9	100%
FEMALE	0	O	FEMALE	0	0
AGE	**NUMBER**	**MEAN±SD**	**AGE**	**NUMBER**	**MEAN±SD**
20-30	8	25.62±2.56	20-30	6	24.23±3.45
31-40	14	35.1±3.45	31-40	2	32.20±2.47
41-50	3	45.5 ± 4.67	41-50	1	41.0±0.00
EDUCATIONAL SATUS	**NUMBER**	**PERCENTAGE**	**EDUCATIONAL STAUS**	**NUMBER**	**PERCENTAGE**
None	12	48%	None	0	0%
Primary	8	32%	Primary	2	8%
Secondary	3	8%	Secondary	2	8%
Tertiary	2	8%	Tertiary	5	55.5%
SOMKING STATUS	**NUMBER**	**PERCENTAGE**	**SOMKING STATUS**	**NUMBER**	**PERCENTAGE**
YES	0	0	YES	0	0
NO	25	100%	NO	9	100%
ALLEGIES	**NUMBER**	**PERCENTAGE**	**ALLEGIES**	**NUMBER**	**PERCENTAGE**
YES	0	0	YES	0	0
NO	25	100	NO	9	100

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4.2 Distribution Of Nuclear Abberations In Buccal Cells Of Exposed And Control Groups

Table 4.2a and Table 4.2b present the distribution of nuclear aberrations observed in buccal epithelial cells obtained from both the right and left cheeks of the exposed and control groups respectively. The analysis was carried out to determine and compare the frequency of nuclear abnormalities between the two groups thereby assessing the extent of genotoxic effects associated with exposure. For the exposed group, 2 samples were collected from both cheeks each to ensure accuracy and to account for any possible differences. Similarly, samples from the control group were analyzed following the same procedures to maintain uniformity in sample collection and analysis. The tables shows the number and types of nuclear abberations identified, providing a clear comparison between the exposed individuals and their unexposed counterparts. this data serves as an important indicator of the genotoxic impact of vehicular emissions on the buccal mucosa of the exposed subject.Table 4.2c Gives the sum total of nuclear abberations gotten from the samples and control.

TABLE 4.2a: Nuclear abberation results obtained from exposed groups.

S/N	LABEL	MN	BN	AN	TOTAL
1	R1	0	0	O	O
	R2	0	1	2	3
	L1	1	1	0	2
	L2	2	0	1	3
	TOTAL	3	2	3	
2	R1	1	0	1	2
	R2	0	0	3	3
	L1	0	1	0	1
	L2	1	1	0	2
	TOTAL	2	2	4	
3	R1	1	0	2	3
	R2	0	1	1	2
	L1	0	1	1	2
	L2	0	1	1	2
	TOTAL	1	3	5	
4	R1	2	0	1	3

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	R2	0	1	0	1
	L1	1	0	1	2
	L2	0	1	1	2
	TOTAL	3	2	3	
5	R1	2	0	2	4
	R2	1	0	0	1
	L1	0	1	1	2
	L2	0	1	0	1
	TOTAL	4	1	3	
6	R1	2	0	0	2
	R2	1	0	0	1
	L1	O	1	1	2
	L2	0	0	0	0
	TOTAL	1	0	1	
7	R1	0	0	0	0
	R2	0	0	1	1
	L1	1	0	1	1

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	TOTAL	1	2	3	
8	R1	1	0	0	1
	R2	0	1	1	2
	L1	0	0	2	2
	L2	0	0	0	0
	TOTAL	1	1	3	
9	R1	1	0	0	1
	R2	0	0	1	1
	L1	0	1	2	3
	L2	1	0	0	1
10	R1	1	1	1	3
	R2	0	1	0	1
	L1	1	1	0	2
	L2	1	0	0	1
	TOTAL	4	3	2	
11	R1	1	0	1	2
	R2	2	1	1	4

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	L1	1	0	1	2
	L2	0	0	0	0
	TOTAL	4	3	3	
12	R1	0	0	1	1
	R2	0	1	2	3
	L1	1	0	0	1
	L2	0	1	0	1
	TOTAL	1	2	3	
13	R1	1	0	1	2
	R2	1	0	0	1
	L1	1	1	0	2
	L2	2	1	0	3
	TOTAL	5	2	1	
14	R1	1	0	1	2
	R2	0	0	1	1
	L1	2	0	1	3
	L2	0	2	0	2

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	TOTAL	3	2	3	
15	R1	1	0	1	2
	R2	1	0	1	2
	L1	2	1	0	3
	TOTAL	5	2	2	
16	R1	0	1	2	3
	R2	0	0	1	1
	L1	2	1	0	3
	L2	1	1	0	2
	TOTAL	1	3	4	
17	R1	1	0	1	2
	R2	0	1	1	2
	L1	1	0	1	2
	L2	0	2	0	2
	TOTAL	2	3	2	
18	R1	0	1	0	1
	R2	0	0	1	1

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	L1	2	1	0	3
	L2	1	0	2	3
	TOTAL	4	1	4	
19	R1	0	1	0	1
	R2	1	3	1	5
	L1	0	0	1	1
	L2	0	0	1	1
	TOTAL	3	4	3	
20	R1	0	0	0	0
	R2	0	0	2	2
	L1	2	1	0	3
	L2	0	1	1	2
		1	3	1	
21	R1	0	0	1	1
	R2	1`	1	0	2
	L1	1	0	1	2

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	L2	0	1	2	3	Formatted[ASUS]: Space Before: 0 pt
	TOTAL	2	2	4		Formatted[ASUS]: Space Before: 0 pt
22	R1	1	1	0	2	Formatted[ASUS]: Space Before: 0 pt
	R2	0	0	2	2	Formatted[ASUS]: Space Before: 0 pt
	L1	0	1	0	1	Formatted[ASUS]: Space Before: 0 pt
	L2	0	1	0	1	Formatted[ASUS]: Space Before: 0 pt
	T0TAL	1	3	2		Formatted[ASUS]: Space Before: 0 pt
23	R1	0	1	1	2	Formatted[ASUS]: Space Before: 0 pt
	R2	0	0	0	0	Formatted[ASUS]: Space Before: 0 pt
	L1	1	0	0	1	Formatted[ASUS]: Space Before: 0 pt
	L2	2	1	1	4	Formatted[ASUS]: Space Before: 0 pt
	TOTAL	3	2	2		Formatted[ASUS]: Space Before: 0 pt
24	R1	0	1	1	2	Formatted[ASUS]: Space Before: 0 pt
	R2	0	1	1	2	Formatted[ASUS]: Space Before: 0 pt
	L1	2	0	1	3	Formatted[ASUS]: Space Before: 0 pt
	L2	1	0	0	1	Formatted[ASUS]: Space Before: 0 pt
25	R1	0	1	1	2	Formatted[ASUS]: Space Before: 0 pt

	R2	1	0	1	2
	L1	1	1	0	2
	L2	0	0	1	1
	TOTAL	2	2	3	

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TABLE 4.2b : Nuclear abberation results obtained from control group.

S/N	LABEL	MN	BN	AN	TOTAL
1	R1	0	0	0	0
	R2	0	1	0	1
	L1	0	1	0	1
	L2	0	0	1	1
	TOTAL	0	2	1	
2	R1	0	0	0	0
	R2	1	1	0	2
	L1	1	0	0	1
	L2	0	0	0	0
	TOTAL	2	1	0	

3	R1	1	0	0	1
	R2	0	0	0	0
	L1	0	0	1	1
	L2	0	0	0	0
	TOTAL	1	0	1	
4	R1	0	0	0	0
	R2	0	0	1	1
	L1	0	0	0	0
	L2	0	0	0	0
	T0TAL	0	0	1	1
5	R1	0	0	0	0
	R2	0	1	0	1
	L1	0	0	0	0
	L2	0	0	1	1
	TOTAL	0	1	1	
6	R1	0	0	0	0
	R2	0	0	1	1

	L1	1	0	0	1
	L2	0	0	0	0
	TOTAL	1	0	1	
7	R1	0	0	0	0
	R2	0	0	1	1
	L1	1	0	1	2
	L2	0	2	1	3
	TOTAL	1	2	3	
8	R1	1	0	0	1
	R2	0	1	1	2
	L1	0	0	2	2
	L2	0	0	0	0
9	R1	3	0	0	3
	R2	0	0	1	1
	L1	0	1	0	1
	L2	1	0	1	2
	TOTAL	4	1	2	

Table 4.2c : The sum total of nuclear aberration gotten from the sample and control.

	MN	BN	An
Sample	73	53	67
Control	10	10	11

Key

S/N= Volunteer sample number

MN= Micronucleus

BN= Binucleated

AN= Anucleated

R1= Right buccal cavity 1

R2= Right buccal cavity 2

L1= Left buccal cavity 1

L2= Left buccal cavity 2

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4.3 Mean Frequencies of Micronucleus , Binucleated and Anucleated Cells in Exposed and Contols Groups With Correspoding P Values From Independent T Test Analysis (SPSS Output)

Table 4.3 presents the mean frequencies of micronucleated, binucleated, and anucleated cells observed in both the sample and control groups. The mean values for the sample group were recorded as follows: micronucleus (2.96 ± 1.37), binucleated (2.12 ± 0.83), and anucleated (2.80

± 0.95). In comparison, the control group showed mean values of micronucleus (1.11 ± 1.20), binucleate (1.22 ± 0.44), and anucleated (1.25 ± 1.20). Furthermore, the p-values obtained from independent t-test analysis using SPSS were 0.004476, 0.004426, and 0.000388 for micronucleus, binucleated, and anucleated respectively. These values indicate a statistically significant difference between the means of the sample and control groups, as all p-values are less than the alpha level of 0.05.

TABLE 4.3: Mean frequency of nuclear abberations of sample and control.

EXPOSED				CONRTROL		
	characteristics	Number	Mean±SD	Number	Mean ± SD	P values
	MN	73	2.96 ± 1.37	10	1.11±1.2	0.004476
	BN	53	2.12 ± 0.83	10	1.22±0.44	0.004422632
	AN	67	2.80±0.95	11	1.25±1.20	0.000388

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4.4 Histogram Representation Of Nuclear Aberration Frequency In Exposed And Control Groups

Figure 4.1 illustrates the histogram representation of the frequency and distribution of nuclear aberrations observed both in the sample and control groups, the chart provides a comparative visualization of the extent of nuclear damage , highlighting variations in the occurences of micro nuclei and other nuclear anomalies between the exposed (sample) and unexposed (control) populations . This graphical representation serves to emphasize differences in genotoxic response , thereby aiding in the assessment of the potential impact of vehicular emissions on cellular integrity.

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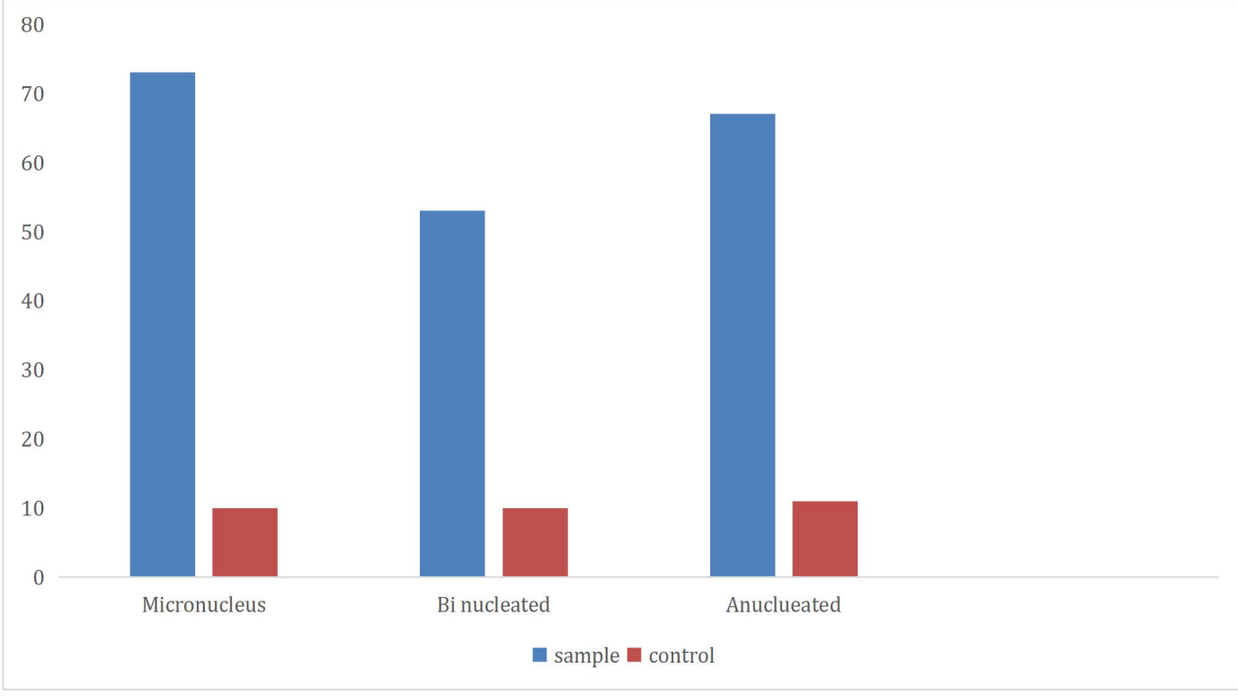

Figure 4.1 : Graphical representation of nuclear aberration in sample

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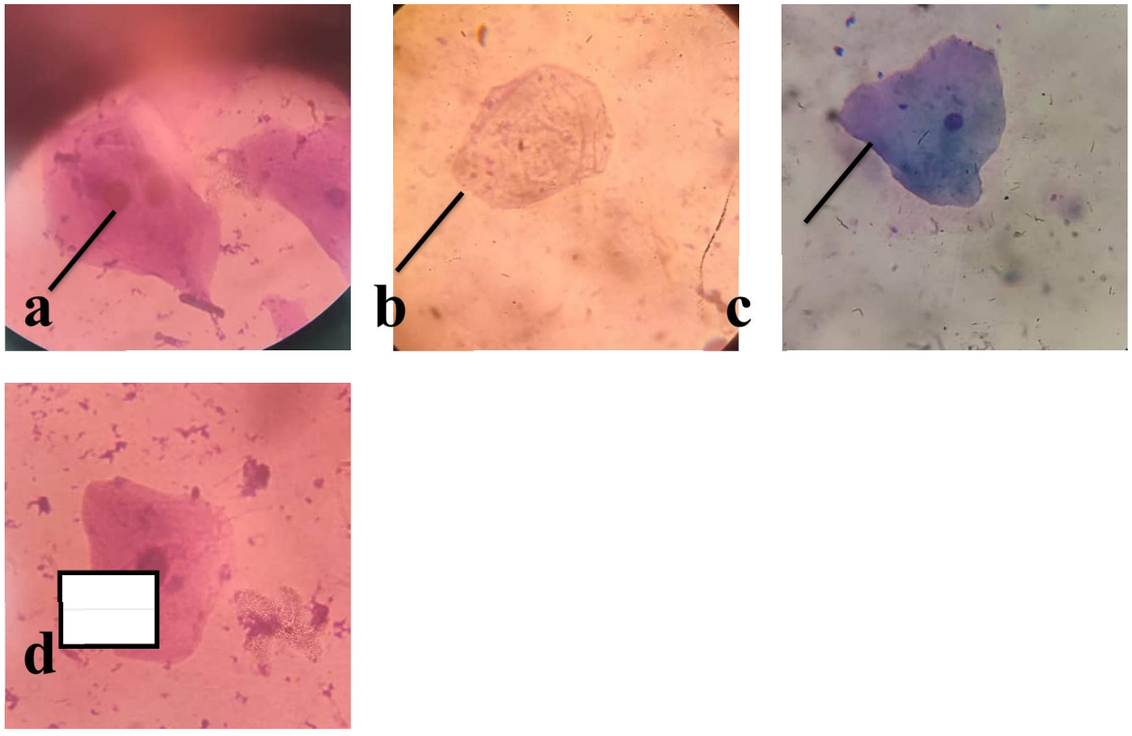

Plate 4.1: Micronuclei induced in exfoliated buccal cells a. Binucleated cell; b. Anucleated cell; c. Normal cell; d. Micronucleus cell

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

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The determination of human, environmental and occupational exposure to toxic chemicals is a fundamental aspect of environmental health research and public safety. Such evaluations are primarily achieved through biomonitoring tools, which enable the assessment of temporal and spatial variations in the burden of contaminants within exposed populations (Alabi *et al.*, 2019). Among these biomonitoring approaches, the buccal micronucleus (MN) cytome assay has gained wide recognition as a robust, sensitive, and cost-effective technique for assessing human exposure to genotoxic agents (kaddah *et al.,* 2022). This assay provides an efficient means of detecting chromosomal damage, genome instability, and cytotoxic effects in exfoliated buccal cells, which are easily obtained through non-invasive sampling procedures (Mladinic *et al.,* 2022). In this study, we investigated the genotoxic effects of vehicular emissions on a group of occupationally exposed individuals—taxi drivers—by applying the micronucleus assay to exfoliated buccal epithelial cells. The findings of the present research reveal a marked increase in the frequency of nuclear abnormalities, including micronucleated, binucleated, and anucleated cells, among the exposed taxi drivers compared to the control group. The observed increase in nuclear aberrations indicates that chronic exposure to vehicular emissions poses a substantial genotoxic risk to individuals who spend prolonged periods in traffic congested urban environments. This result is consistent with previous studies that have identified vehicular emissions as a major source of genotoxic and cytotoxic air pollutants (Lewtas, 2007). Vehicular emissions contain a complex mixture of particulate matter (PM), nitrogen oxides (NOx), carbon monoxide (CO), volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons

(PAHs), many of which are known or suspected carcinogens and mutagens (Monks *et al.,* 2012). Continuous inhalation and dermal contact with these pollutants can result in their bioaccumulation within human tissues, where they interact with cellular macromolecules, including DNA. This interaction may induce oxidative stress, DNA strand breaks, chromosomal mis-segregation, and the formation of micronuclei. The elevated frequency of micronuclei observed in the buccal cells of taxi drivers therefore reflects an ongoing process of genotoxic insult and genomic instability resulting from occupational exposure to these toxicants. Previous epidemiological and toxicological studies have consistently demonstrated the health risks associated with long-term exposure to traffic related air pollution. Smith *et al.*, (2017) reported significant associations between maternal exposure to vehicular pollutants and adverse birth outcomes, including low birth weight and preterm delivery. Similarly, Mudway *et al.,* (2019) observed that chronic exposure to particulate matter and gaseous pollutants impairs lung development in children, leading to long-term respiratory dysfunction. Pedrerol *et al.,* (2017) further established links between early life exposure to traffic related air pollutants and impaired cognitive development, suggesting that these pollutants may interfere with neurodevelopmental processes. At the adult level, long-term exposure to vehicular emissions has been linked to a variety of chronic and degenerative diseases. Epidemiological data show a strong correlation between sustained pollutant exposure and increased incidence of chronic respiratory illnesses, including asthma, chronic obstructive pulmonary disease (COPD), and bronchitis (Gehring *et al.,* 2015; Pfeffer *et al.*, 2018; Samoli *et al.*, 2016). Moreover, several studies have demonstrated that inhalation of fine and ultrafine particles contributes to endothelial dysfunction and systemic inflammation, key mechanisms underlying cardiovascular disease development (Alexeeff *et al.*, 2018; Atkinson *et al.*, 2010; Bell *et al.*, 2014). Beyond respiratory and cardiovascular effects,

mounting evidence points to the neurological and carcinogenic consequences of prolonged exposure to vehicular pollutants. Carey *et al.,* (2016) observed an increased risk of dementia among individuals residing in high traffic areas, implicating oxidative stress and neuroinflammation as potential mediators. Hart *et al.,* (2015) reported elevated incidences of various cancers including lung, bladder, and skin cancer among populations chronically exposed to traffic emissions. Likewise, Atkinson *et al.* (2016)., and Hoek *et al.*, (2002) demonstrated a clear association between long-term exposure to fine particulate matter and premature mortality, underscoring the grave public health implications of vehicular pollution. The increased frequency of nuclear aberrations observed in taxi drivers compared to the control group in this study may be directly attributed to their prolonged occupational exposure to vehicular exhaust. Taxi drivers typically spend extended hours on the road, often in congested areas where pollutant concentrations are highest. The nature of their work subjects them to cumulative inhalation of exhaust fumes, especially during traffic jams, idling, and in poorly ventilated vehicles. Such chronic exposure can overwhelm cellular antioxidant defenses, leading to oxidative DNA damage, impaired repair mechanisms, and genomic instability key hallmarks of genotoxic stress.

5.2 Conclusion

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The findings of this study provide compelling evidence that vehicular emissions pose significant genotoxic risks to drivers who are consistently exposed to traffic-related pollutants. Continuous occupational exposure to vehicular exhaust particularly among commercial drivers, traffic wardens, and other road users results in the accumulation of harmful substances such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, benzene, and fine particulate matter ($PM_{2.5}$ and PM_{10}). These compounds are capable of penetrating biological membranes, generating reactive oxygen species (ROS), and interacting directly with cellular DNA, leading to

genetic instability and chromosomal damage. The elevated frequency of micronuclei, binucleated cells, and other nuclear abnormalities observed among exposed individuals compared to the control group reflects a clear indication of genotoxic insult. These biomarkers serve as early warning indicators of potential long-term health risks, including carcinogenesis, neurotoxicity, and reproductive impairment. The buccal micronucleus cytome assay used in this study proved to be a sensitive and non-invasive tool for monitoring such genotoxic alterations in human populations, emphasizing its importance in environmental and occupational health assessments. The data underscore the urgent need for stricter environmental policies and enforcement of vehicular emission control standards. Regular maintenance of vehicles, the adoption of cleaner fuels and engines, and the promotion of alternative transportation systems can greatly minimize exposure. Furthermore, drivers should be encouraged to undergo periodic biomonitoring evaluations and adopt personal protective strategies such as ensuring proper cabin ventilation and limiting idling time to reduce inhalation of exhaust fumes.

In conclusion, vehicular emissions represent a major environmental and occupational health threat capable of inducing genetic damage in chronically exposed drivers. Addressing this issue requires a multidimensional approach that integrates scientific monitoring, public health awareness, and sustainable transport policies. Through concerted action, it is possible to safeguard not only the genetic integrity of vulnerable populations but also the broader environmental quality essential for human well-being and future generations.

5.3 RECOMMENDATION

1. Regular Biomonitoring of Exposed Workers: Implement periodic micronucleus and cytogenetic assays among commercial drivers and traffic personnel to detect early genotoxic damage caused by vehicular emissions.

2. Mandatory Health Screening: Establish routine medical and genetic health checks for drivers, focusing on early signs of DNA damage, respiratory distress, and oxidative stress.

3. Strengthened Air Quality Regulations: Enforce strict emission control laws and regular inspection of vehicles to minimize the release of carcinogenic and genotoxic pollutants.

4. Promotion of Eco-Friendly Transport: Encourage the adoption of low-emission, hybrid, or electric vehicles, especially for public and commercial transportation within city centers.

5. Creation of Green Buffer Zones: Plant trees and maintain green belts around major motor parks to absorb particulate matter and reduce exposure to airborne pollutants.

6. Occupational Protection Measures: Provide protective face masks or filters for drivers and traffic workers to reduce inhalation of vehicular fumes, especially during peak hours.

7. Exposure Time Regulation: Regulate working hours and shift durations for drivers exposed to high-traffic zones to minimize cumulative exposure to emissions.

8. Public Health Awareness Programs: Conduct educational campaigns to sensitize drivers and the public on the health risks of vehicular emissions and the importance of personal protective measures.

9. Smoking Cessation Enforcement: Reinforce no-smoking policies among drivers participating in biomonitoring studies to eliminate confounding factors that enhance genotoxicity.

10. Mental and Cognitive Health Monitoring: Include neurobehavioral assessments in long-term studies since genotoxic damage is often linked to cognitive decline and neurodegenerative risks.

11. Establish Mobile Health Units: Deploy mobile health clinics in high-traffic areas to conduct onsite biomonitoring, sample collection, and health education for occupationally exposed workers.

12. Expand Research on Molecular Mechanisms: Encourage molecular-level studies (e.g., DNA adduct analysis, oxidative stress markers) to understand the pathways of vehicular emission–induced genotoxicity.

13. Integration into Environmental Policy: Incorporate biomonitoring data from such studies into national environmental and occupational health policies for data driven decision making.

14. Longitudinal Follow-Up Studies: Conduct long-term cohort studies to observe how continued exposure over years affects genomic stability and disease outcomes.

15. Control of Fuel Quality: Implement strict quality control measures for fuels to reduce sulfur, lead, and polycyclic aromatic hydrocarbon (PAH) content that contribute to genotoxic effects.

16. Comparative Regional Studies: Replicate similar genotoxicity studies in different cities or traffic zones to establish nationwide data on vehicular emission–related DNA damage.

17. Vehicle Maintenance Enforcement: Mandate regular vehicle servicing and emissions testing for all public transport vehicles to ensure compliance with emission standards.

18. Genetic Susceptibility Research: Investigate genetic polymorphisms (e.g., detoxification enzyme genes) among drivers to identify those more vulnerable to genotoxic effects.

19. Inclusion in Academic Curricula: Integrate environmental genotoxicology and occupational health into university and driver training curricula to improve awareness and prevention.

20. Collaboration Between Agencies: Promote intersectoral collaboration between environmental agencies, health ministries, and transport unions to ensure sustained monitoring and intervention programs.

REFERENCES

Alabi, O.A., Adeoluwa, Y.M. and Bakare, A.A.,(2020). Elevated serum Pb, Ni, Cd, and Cr levels and DNA damage in exfoliated buccal cells of teenage scavengers at a major electronic waste dumpsite in Lagos, Nigeria. *Biological Trace Element Research, **I**94*(1):24-33.

Alexeeff SE, Roy A, Shan J, Liu X, Messier K, Apte JS, Portier C, Sidney S, Van Den Eeden SK. (2018). High-resolution mapping of traffic related Air pollution with Google street view cars and incidence of cardiovascular events within neighborhoods in Oakland, CA. *Environmental Health* **17**(1):38-47.

Atkinson RW, Analitis A, Samoli E, Fuller GW, Green DC, Mudway IS, Anderson HR, Kelly FJ (2016) Short-term exposure to trafficrelated air pollution and daily mortality in London, UKJ *Journal of Exposure Science & Environmental Epidemiology*. **26(**2):125–132.

Atkinson RW, Fuller GW, Anderson HR, Harrison RM, Armstrong B.(2010). Urban ambient particle metrics and health: a time-series analysis. *Epidemiology (Cambridge, Mass)* **21**(4):501–511.

Baptista, F., Garcia, P.V., Rodrigues, A.S. and Ladeira, C., (2025). Genotoxicity and Cytotoxicity Assessment of Volatile Organic Compounds in Pathology Professionals Through the Buccal Micronuclei Assay. *Toxics*, **13**(5): 411- 415.

Bell ML, Ebisu K, Leaderer BP, Gent JF, Lee HJ, Koutrakis P, Wang Y, Dominici F, Peng RD .(2014). Associations of PM2.5 constituents And sources with hospital admissions: analysis of four counties in Connecticut and Massachusetts (USA) for persons ≥ 65 years of age. *Environment Health perspective **I**22*(2):138–144.

Cames M, Helmers E. (2013). Critical evaluation of the European diesel Car boom—Global comparison, environmental effects and various National strategies. *Environment Science Europe* **25**(15): 1-7

Carey IM, Anderson HR, Atkinson RW, Beevers S, Cook DG, Dajnak D,Gulliver J, Kelly FJ (2016) Traffic pollution and the incidence of Cardiorespiratory outcomes in an adult cohort in London. *Occupational Environmental Medicine* .**73**(12):849–856.

European Environment Agency. (2018). Dieselisation (share of diesel Cars in the total passenger car fleet) [Data Visualization]. European Environment Agency. https://www.eea.europa.eu/dataand-maps/daviz/dieselisation-of-diesel-cars-in-4#tab-chart_1

Gehring U, Wijga AH, Hoek G, Bellander T, Berdel D, Brüske I, Fuertes E, Gruzieva O, Heinrich J, Hoffmann B, de Jongste JC, Klümper C, Koppelman GH, Korek M, Krämer U, Maier D, Melén E, Pershagen G, Postma DS, Standl M, von Berg A, Anto JM, Bousquet J, Keil T, Smit HA, Brunekreef B. (2015). Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: a population-based birth cohort study. *Lancet Respiratory Medicine*, **3**(12):933–942.

Hart JE, Spiegelman D, Beelen R, Hoek G, Brunekreef B, Schouten LJ, Brandt P .(2015). Long-term ambient residential traffic-related exposures and measurement error-adjusted risk of incident lung cancer in the Netherlands cohort study on diet and cancer. *Environment Health Perspective.* **123**(9):860–866

Health Effects Institute. (2010). Traffic-related air pollution: a critical Review of the literature on emissions, exposure, and health effects. **17**: 386-390.

Hoek G, Brunekreef B, Goldbohm S, Fischer P, van den Brandt PA. (2002). Association between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study. *Lancet* **360**(9341):1203–1209.

Kadeh, H., Saravani, S., Moradi, M. and Alimanesh, N.,(2023). A comparative evaluation of the genotoxic effects of mobile phone radiation using buccal micronucleus assay. *Journal of Dentistry*, **24**(1): 118- 125.

Lewtas, J., (2007). Air pollution combustion emissions: characterization of causative Agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. journal series ***journal series Mutation Research*** **636, 95–133.**

Luan, Y. and Honma, M., (2022). Genotoxicity testing and recent advances. *Genome Instability & Disease, **3***(1): 1-21.

Mohamed, S.A.K.S., Sabita, U., Rajendra, S. and Raman, D., (2017). Genotoxicity: Mechanisms, testing guidelines and methods. *Global Journal of Pharmacy & Pharmaceutical* Sciences, **1**: 133-138

Mudway IS, Dundas I, Wood HE, Marlin N, Jamaludin JB, Bremner SA,Cross L, Grieve A, Nanzer A, Barratt BM, Beevers S, Dajnak D, Fuller GW, Font A, Colligan G, Sheikh A, Walton R, Grigg J, Kelly FJ, Lee TH, Griffiths CJ .(2019). Impact of London's low emission Zone on air quality and children's respiratory health: a sequential Annual cross-sectional study. *Lancet Public Health* , **4**(1) :28–40.

Olorunfemi, D.I., Orororo, O.C., Iloduba, N.E., Osioma, E., Kpomah, E.D. and Osio, O.L., (2024). Evaluation of Genotoxicity by Comet assay in tissues of Clarias gariepinus

exposed to cassava Effluent. *Asian Journal of Biochemistry, Genetics and Molecular Biology,* **16**(7) : 98-108

Pagaddu, J.V.A., Martinez, M., Ruiz, E.R., Briosos, H., Baculi, R., Alias, L., Cruz, J.L.D. and Benito, R., (2021). Genotoxic Effect of Automobile Exhaust Exposure among Motorized Tricycle Drivers in Tuguegarao City, Cagayan, Philippines using Micronucleus Assay: A Retrospective Cohort Study. *International Journal of Environment, Agriculture and Biotechnology*, **6**(1): 120-126.

Pedrerol M, Rivas I, López-Vicente M, Suades-González E, Donaire-Gonzalez D, Cirach M, de Castro M, Esnaola M, BasagañaX, Dadvand P, Nieuwenhuijsen M, Sunyer J (2017) Impact of commuting exposure to traffic-related air pollution on cognitive development inChildren walking to school. *Environmental Pollution*, **231** :837–844.

Pfeffer PE, Donaldson GC, Mackay AJ, Wedzicha JA. (2018) .Increased Chronic obstructive pulmonary disease exacerbations of likely viral etiology follow elevated ambient nitrogen oxides. *American Journal of Respiratory and Critical Care Medicine*. **199**(5):581–591.

Risom, L., Møller, P., Loft, S.,(2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research* , **592** : 119–137.

Romieu, I., Castro-Giner, F., Kunzli, N., Sunyer, J.,(2008). Air pollution, oxidative stress And dietary supplementation: a review. ***European Respiratory Journal***. **31**: 179–196.

Samoli E, Atkinson RW, Analitis A, Fuller GW, Green DC, Mudway I, Anderson HR, Kelly FJ. (2016) .Associations of short-term exposure To traffic-related air pollution with cardiovascular and respiratory Hospital admissions in London, UK. *Occupational Medicine and Environment* **73**(5): 300–307.

Samoli E, Atkinson RW, Analitis A, Fuller GW, Green DC, Mudway I, Anderson HR, Kelly FJ .(2016) .Associations of short-term exposure To traffic-related air pollution with cardiovascular and respiratory Hospital admissions in London, UK. Occupational Environmental Medicine **73**(5): 300–307.

Smith RB, Fecht D, Gulliver J, Beevers SD, Dajnak D, Blangiardo M,Ghosh RE, Hansell AL, Kelly FJ, Anderson HR, Toledano MB.(2017) .Impact of London's road traffic air and noise pollution on Birth weight: retrospective population based cohort study. *Toxicology and Applied Pharmacology.* **206**, 73–93.

Snyder, R., Hedli, C.C., 1996. An overview of benzene metabolism. *Toxi*cology *and Applied pharmacology.* **104** (6) : 1165–1171.

Valavanidis, A., Fiotakis, K., Vlachogianni, T., (2008). Airborne particulate matter and Human health: toxicological assessment and importance of size and compositionOf particles for oxidative damage and carcinogenic mechanisms. *Journal of Environmental Science and Health,* **26**: 339–362.

Vassoler, T., Dogenski, L.C., Sartori, V.K., Presotto, J.S., Cardoso, M.Z., Zandoná, J., Trentin, M.S., Linden, M.S., Palhano, H.S., Vargas, J.E. and De Carli, J.P., (2021). Evaluation of the genotoxicity of tobacco and alcohol in oral mucosa cells: a pilot study. *The Journal of Contemporary Dental Practice,* **22**(7): 745-750.

Xue, W., Warshawsky, D., 2005. Metabolic activation of polycyclic and heterocyclic Aromatic hydrocarbons and DNA damage: a review. *Toxicology and Applied Pharmacology*. **206**, 73–93

Yang, W., Omaye, S.T., (2009). Air pollutants, oxidative stress and human health.*journal series Mutation Research,* **674**, 45–54.

APPENDIX

University of Benin, Benin City, Nigeria

DEPARTMENT OF ENVIRONMENTAL MANAGEMENT & TOXICOLOGY
FACULTY OF LIFE SCIENCES

VOLUNTEER DEMOGRAPHIC INFORMATION FORM (SAMPLE COLLECTION)

Personal Information

1. Full Name: _____

2. Date of Birth: _____/_____/_____
(DD/MM/YYYY)

3. Gender:
[ ] Male [ ] Female [ ] Non-binary [ ] Prefer not to say [ ] Other: _____

4. Contact Information:
Phone: _____ Email: _____

Address: _____

Demographic Details

5. Ethnicity/Race: (Optional)
[ ] Bini [ ] Esan [ ] Ibo [ ] Etsako [ ] Yoruba [ ] Others
[ ] Prefer not to say

6. Highest Education Level
[ ] None [ ] Primary [ ] Secondary [ ] Tertiary [ ] Others

Health & Lifestyle Information
8. Smoking Status: [ ] Smoker (Current) [ ] Former Smoker [ ] Non-smoker

9. Pregnancy Status: (If applicable) [ ] Pregnant [ ] Not Pregnant [ ] Not Applicable

10. Do you have any known allergies or medical conditions?
[ ] Yes (Specify: _____) [ ] No

CONSENT AND AGREEMENT

11. Consent for Data Use: I agree that my anonymized demographic data may be used for research purposes.
[ ] I consent [ ] I do not consent

Volunteer Signature: _____

Date: _____/_____/_____