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Review Article

Potential Genotoxic Effects of Waterpipe Smoking on Human Health: Lessons Learnt from Lab and Field studies

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ABSTRACT Waterpipe smoking is widely considered as safer alternative to cigarette smoking due to its filtration through water. However, recent studies suggested that waterpipe smoke may have significant genotoxic effects that could lead to different diseases. This rapid review aims to identify the effects of genotoxicity of waterpipe smoking. A systematic literature search was conducted in PubMed, SCOPUS, and Google Scholar, following PRISMA guidelines. Studies were included if they investigated DNA damage, chromosomal changes etc associated with waterpipe smoking in human studies and laboratory models (in vitro and in vivo). Of total 211 studies searched, a total of 27 studies met the inclusion criteria. Most included studies were in vitro (cellular models) and in vivo (animal models), while fewer studies were based on human observations. Commonly reported markers included 8-OHdG and MDA for oxidative stress, and IL-6 and TNF-α for pro-inflammatory response. Network analysis revealed clusters related to genotoxicity markers, heart, bone marrow, lungs etc. These findings suggest a robust mechanistic basis for waterpipe smoke-induced genotoxicity in laboratory settings, though further human-based research is warranted to confirm these effects. This review consolidates evidence of genotoxic effects from waterpipe smoking, demonstrating that oxidative stress and inflammation play critical roles in mediating DNA damage which will further form basis of organ-associated diseases. Although in vitro and in vivo models provide valuable insights but human-based studies are needed to validate these mechanisms and inform public health strategies.

KEYWORDS Waterpipe Smoking, Genotoxicity, DNA Damage, Oxidative Stress, Inflammatory Markers

Introduction

Waterpipe smoking, also known as hookah, shisha, or narghile, has become increasingly popular as safer alternative of cigarette (Khalil *et al*, 2019; Hamadi *et al*, 2024; Nemmar *et al*, 2024). Waterpipe smoking is traditionally used in Middle Eastern and South Asian cultures but it has now spread to Europe and North America (Kuntz *et al*, 2015; Jebai *et al*, 2021). It is often perceived as less harmful than cigarette smoking due to the filtration of smoke through water. Recent evidences suggest that waterpipe smoking could pose serious health risks associated with respiratory and cardiovascular systems (Aljadani *et al*, 2020). However, there are very few studies related to genotoxic effects of waterpipe smoking.

Genotoxicity is a critical issue in the context of smoking and tobacco (Alkan and Koroglu-Aydin, 2023). It refers to alteration in DNA structure or segregation i.e. DNA fragmentation, DNA migration etc. (Menz *et al*, 2023). Previous studies have demonstrated that cigarette smoke contains harmful compounds that are genotoxic in nature. These effects have been widely studied in both human and animal models (Nemmar *et al*, 2019b). Waterpipe smoking involves tobacco burning and large volumes of smoke generation which contain components that can cause higher levels of DNA damage (Aljadani *et al*, 2020). However, the genotoxic potential of waterpipe smoke remains less understood.

Recently explored genotoxic effects of waterpipe smoking used both human and laboratory-based models such as mice and cell cultures. These lab models allow for controlled experiments to undermine specific mechanisms of DNA

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damage (Beegam *et al*, 2024). Some studies have reported genotoxic effects in human populations (Cetkovic Pecar *et al*, 2023). But lab-based studies provide deeper insights into the biological pathways that could lead to cancer and other health complications. This rapid review aims to synthesize findings from both human and laboratory studies to better understand the genotoxic risks associated with waterpipe smoking and to highlight lessons learned from these lab models that may inform future public health strategies.

Materials and Methods

This rapid review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and reproducibility (Page *et al*, 2021). This study aimed to identify previous studies on the genotoxic effects of waterpipe smoking, specifically targeting DNA damage, chromosomal aberrations, and other genotoxic outcomes. This search focused on human studies, animal models, and cell culture studies that investigated the effects of waterpipe smoking.

Literature Search

The literature search was performed across three electronic databases: PubMed, SCOPUS, and Google Scholar. Search terms included combinations of "waterpipe smoking," "genotoxicity," "DNA damage," "micronucleus formation," and "chromosomal aberrations." A total of 233 records were initially retrieved, with 85 from PubMed, 113 from SCOPUS, and 35 from Google Scholar. After removing 22 duplicate records, 211 unique records were considered for screening (Fig. 1).

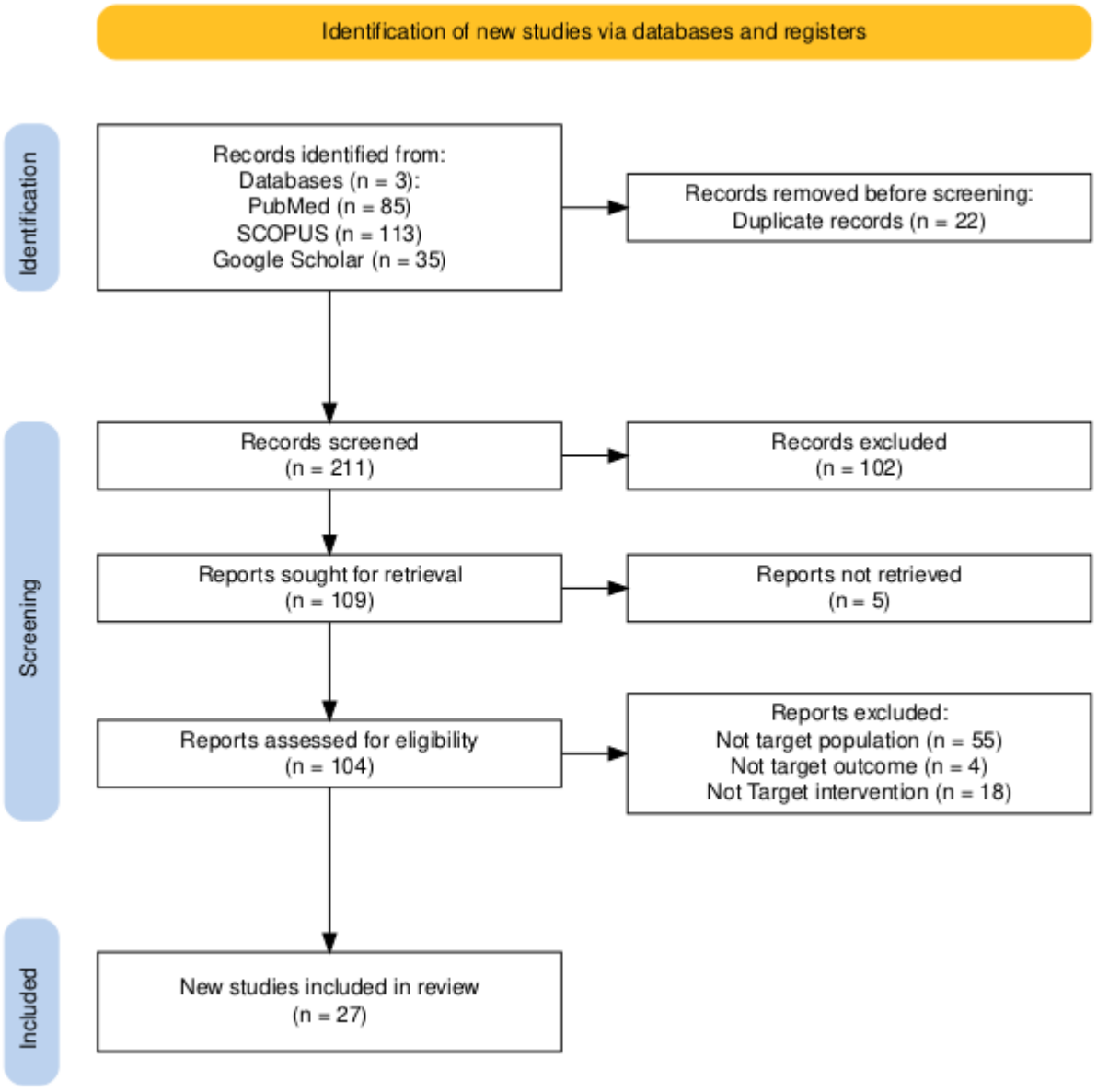

Fig. 1: Identification of studies based on the PRISMA guidelines.

Screening and Eligibility

Titles of the 211 unique records were screened for relevance to the review's inclusion criteria. Studies were included if they involved human participants, mice models, or cell cultures exposed to waterpipe smoking, with specific outcomes related to DNA damage, chromosomal changes, or other genotoxic markers. Of the 211 records screened, 102 were excluded based on irrelevance or lack of alignment with the target population, exposure, or outcome. Title and abstract reviews were sought for 109 articles, and 5 could not be retrieved, resulting in 104 reports assessed for eligibility.

Inclusion and Exclusion Criteria

Out of the 104 abstracts assessed, 77 were excluded: due to multiple reasons. Ultimately, 27 studies met all criteria and were included in the final review. These studies comprised a mix of observational studies, controlled trials, and experimental laboratory models, providing a broad view of the genotoxic effects of waterpipe smoking.

Table 1: Studies included for downstream analysis using PRISMA guidelines.

Sr. No	Study	Year	Subjects	Organs/Tissue/Cells
1.	(Beegam *et al*, 2024)	2024	Mice	Chronic Kidney Disease
2.	(Hamadi *et al*, 2024)	2024	Mice	Cardiac system
3.	(Nemmar *et al*, 2024)	2024	Mice	Liver
4.	(Wise and Hein, 2024)	2024	Cell-line	Bronchial epithelial cells
5.	(Cetkovic Pecar *et al*, 2023)	2023	Human	General systems
6.	(Hamadi *et al*, 2023)	2023	Mice	Cerebellum
7.	(Nemmar *et al*, 2023a)	2023	Mice	Cardiac system
8.	(Nemmar *et al*, 2023b)	2023	Mice	Lungs and Heart
9.	(Nemmar *et al*, 2022)	2022	Mice	Cardiac system
10.	(Jebai *et al*, 2021)	2021	Human	General systems
11.	(Tellez *et al*, 2021)	2021	Cell-line	Epithelial cells and Cardiac systems
12.	(Zaarour *et al*, 2021)	2021	Cell-line	Lungs
13.	(Abi-Gerges *et al*, 2020)	2020	Mice	General systems
14.	(Nemmar *et al*, 2020a)	2020	Mice	Cardiac system
15.	(Nemmar *et al*, 2020b)	2020	Mice	Kidney
16.	(Rajabi-Moghaddam *et al*, 2020)	2020	Cell-line	Buccal exfoliated cells
17.	(Alsaad *et al*, 2019)	2019	Human	General systems
18.	(Khalil *et al*, 2019)	2019	Cell-line	Lungs
19.	(Nemmar *et al*, 2019a)	2019	Mice	Cardiac system
20.	(Nemmar *et al*, 2019b)	2019	Mice	Lungs
21.	(Azab *et al*, 2018)	2018	Mice	Bone-marrow
22.	(Silveira *et al*, 2018)	2018	Human	General system
23.	(Nemmar *et al*, 2017)	2017	Mice	Cardiac system
24.	(Derici Eker *et al*, 2016)	2016	Human	General system
25.	(Nemmar *et al*, 2016)	2016	Mice	Lungs
26.	(Azab *et al*, 2015)	2015	Cell-line	Saliva, Urine, Serum
27.	(Al-Amrah *et al*, 2014)	2014	Human	Buccal epithelial cells and Leukocytes

Comparison of studies

A database was developed using the selected studies in Microsoft Excel spreadsheet. These studies were screened for terms regarding proinflammatory enzymes, oxidative stress, genotoxic techniques, study types, and organs/tissues/cells involved. Comparison of these terms were made to understand the distribution.

Data Extraction and Term Co-occurrence Analysis

To visualize the relationships between key terms and concepts across the included studies, VOS viewer (version 1.6.20), a software tool for constructing and visualizing networks. After selecting the final set of 27 studies, the relevant terms and keywords from titles, abstracts, and keywords sections of each article were imported into VOS viewer for analysis.

Results

Genotoxicity Techniques Across Study Types

Comparison of genotoxicity techniques among types of studies revealed that the Comet Assay was utilized in most studies (Table 2). The 8-OHdG (8-hydroxy-2'-deoxyguanosine) assay, a biomarker for oxidative DNA damage, suggesting moderate attention to oxidative stress assessment in waterpipe-related research. Other techniques, such as Sister Chromatid Exchange (SCE) assay/Chromosomal Aberration Assay and gamma-H2AX protein detection were less frequently used in this area of research.

Table 2: The table summarizes the use of various genotoxicity assessment techniques across human, in vitro, and in vivo studies focused on the effects of waterpipe smoking.

Techniques	Types of Studies		
	Human	In-Vitro	In-Vivo
8-OHdG	2	1	1
BMCyt Assay	1	0	0
Comet Assay	3	2	13
Microscopy	1	1	0
gama-H2Ax protein expression	0	1	0
SCE assay/ C-Aberration Assay	0	0	1

Oxidative Stress and Proinflammatory Markers

Comparison of oxidative stress and proinflammatory markers revealed common use of enzymes for estimating the Reactive Oxygen Species (ROS) and related varying response of body (Fig. 2). The oxidative stress markers are more frequently reported, with the highest number of studies determining Lipid peroxidation (LPO) followed by Glutathione (GSH), and Superoxide Dismutase (SOD). Glutathione Disulfide (GSSG) was the least used marker.

Proinflammatory markers, while less frequently reported, include combinations of Tumor Necrosis Factor-alpha (TNF-α) with other interleukins such as IL-6, IL-1β, and IL-17. The highest number of reports within this category involve studies examining TNF-α alone or in combination with IL-6 and IL-1β.

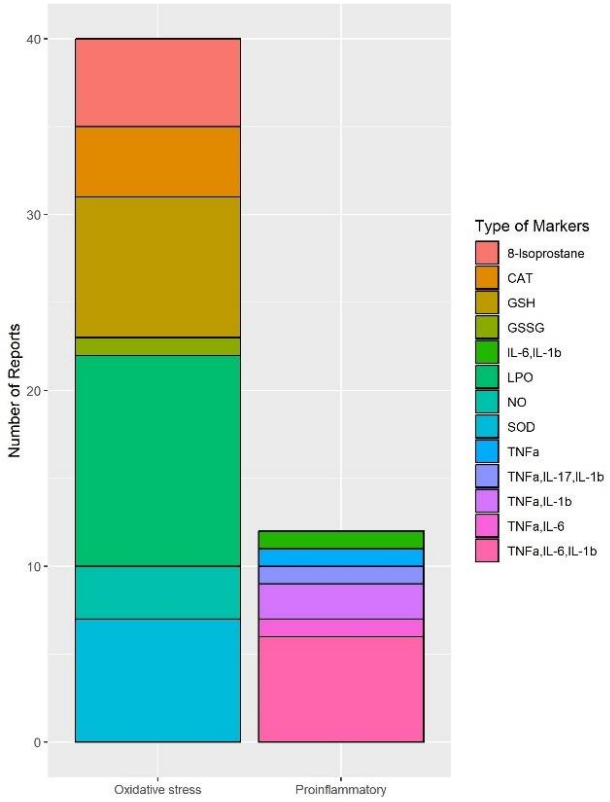

Fig. 2: Distribution of oxidative stress and proinflammatory markers reported in studies assessing the genotoxic effects of waterpipe smoking.

Network Analysis

The VOS viewer network analysis revealed clusters of key terms frequently associated with waterpipe smoking and genotoxicity in the reviewed literature (Fig.3). Terms related to heart or cardiovascular system formed a prominent cluster (Red), indicating a strong association with studies on the genotoxic effects of waterpipe smoking. Another significant cluster (green) involved terms such as waterpipe smoking and genotoxic effects which were often linked to studies investigating pro-inflammatory and oxidative responses to waterpipe smoke exposure.

Another cluster (Blue) revolves around general smoking behavior and its health impacts. Terms like "waterpipe smoking," "nonsmoker," "genotoxic effect," and "cigarette smoking" indicate research comparing waterpipe use to other forms of smoking, as well as exploring the broader genotoxic effects. While the last cluster (yellow) is smaller cluster that contains terms related to tobacco products, such as "tobacco," "cigarillo," "hookah," and "shisha tobacco product." This cluster might reflect studies that compare different tobacco product types and usage behaviours among waterpipe users.

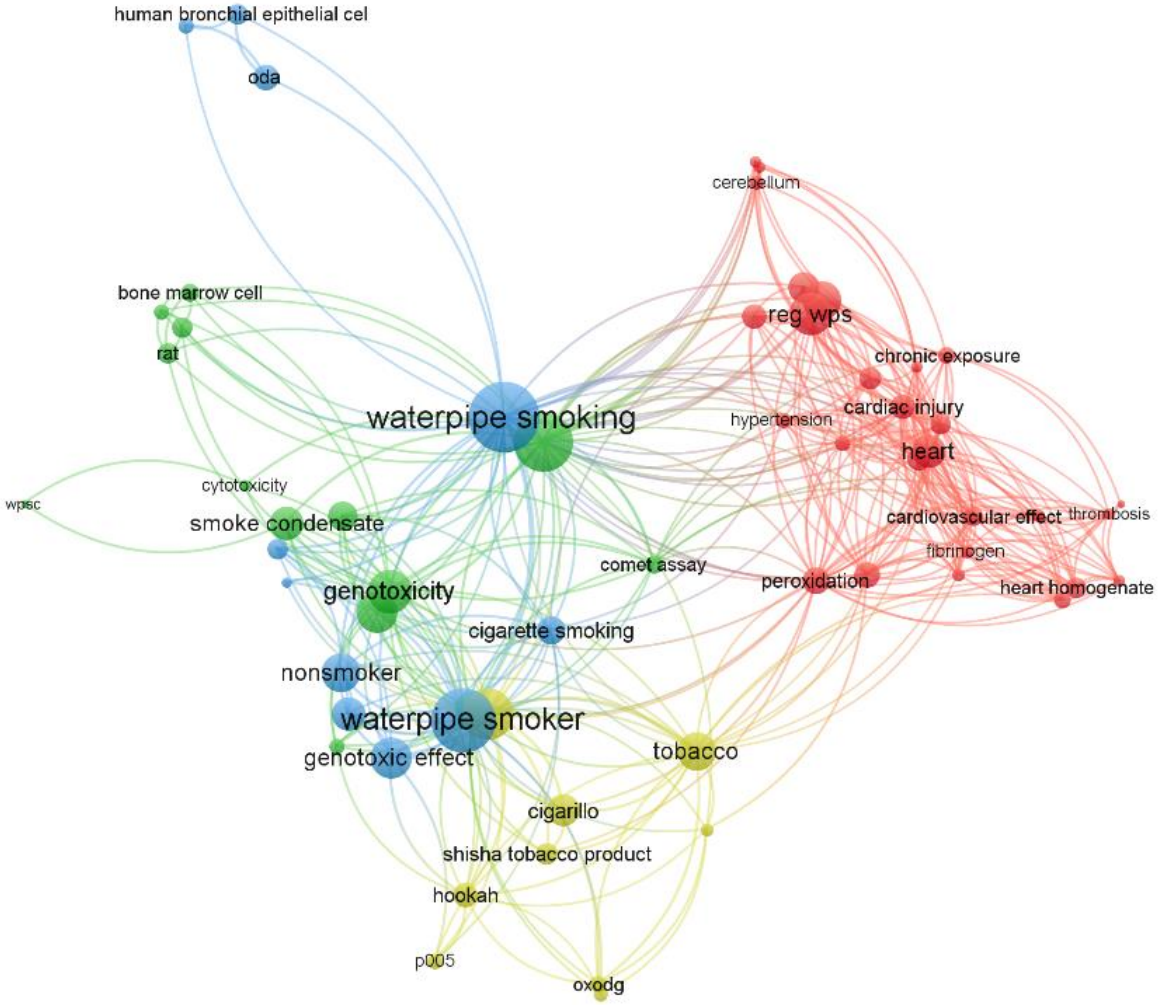

Fig. 3: Network analysis of the terms associated with water pipe smoking. Four clusters are formed.

The most prominent node is "waterpipe smoking," which serves as the central hub for most connections across clusters. The network has strong interconnections, particularly between the red and blue clusters. It indicates that cardiovascular and toxicological studies are often interlinked. Terms like "cardiac injury" and "cytotoxicity" show moderate overlap, suggesting studies exploring both physiological and cellular effects. The density of links within clusters suggested a concentrated research focus on health risks associated with waterpipe smoking. The green cluster also has strong internal links, indicating cohesive research on toxicological effects.

Possible chain of events post-smoking exposure

Exposure to waterpipe smoking, either occasional or regular, initiates pro-inflammatory cytokines which increases the ROS species in the body. Imbalance in ROS species and body responses against ROS leads to oxidative stress. Excessive oxidative stress can cause genotoxicity or DNA damage in the cells/tissues. Genotoxic events can activate the pathways leading to endothelium damage and apoptosis or necrosis. Eventually leading to damage to the studied organs/tissues or cell lines (Fig. 4).

Discussion

Current rapid review identifies the important gaps in the research related to waterpipe smoking and genotoxicity. Identification of studies is followed as previous studies and PRISMA guidelines (da Silva, 2016; Rababa'h *et al*, 2021). This study has more broader research question to address as designated framework. The inclusion criteria led to select 27 research articles which is comparatively high number for independent reviewers in rapid review.

The distribution of techniques mentioned in this study indicates a primary reliance on the Comet Assay for DNA damage assessment. Comet assay has been used for DNA migration and DNA breakage from many years (da Silva, 2016; da Silva *et al*, 2021).This study also indicates that there is need to study the comparative diagnostic markers to understand the sensitivity and specificity of comet assay among in vitro, in vivo and observational studies. Furthermore, it was observed that relatively fewer studies employed cytogenetic techniques, especially in non-human models.

Current study demonstrates a predominant focus on oxidative stress markers in the literature. It also suggests that oxidative damage is a primary pathway studied in the context of waterpipe smoking's genotoxic effects. There is a growing interest in the oxidative stress pathways activated by waterpipe smoking (Allahverdi *et al*, 2021; Novelli *et al*, 2022). Meanwhile, proinflammatory markers are explored to a lesser extent, reflecting an emerging but relatively less established research area.

The map highlights a multidisciplinary approach, combining cardiovascular, toxicological, and comparative smoking studies. The presence of terms like "chronic exposure" and "genotoxic effect" underscores the growing interest in the long-term health impacts of waterpipe smoking. The visibility of "cigarette smoking" as a node suggests a comparative angle, with researchers examining differences and similarities between waterpipe smoking and cigarette use.

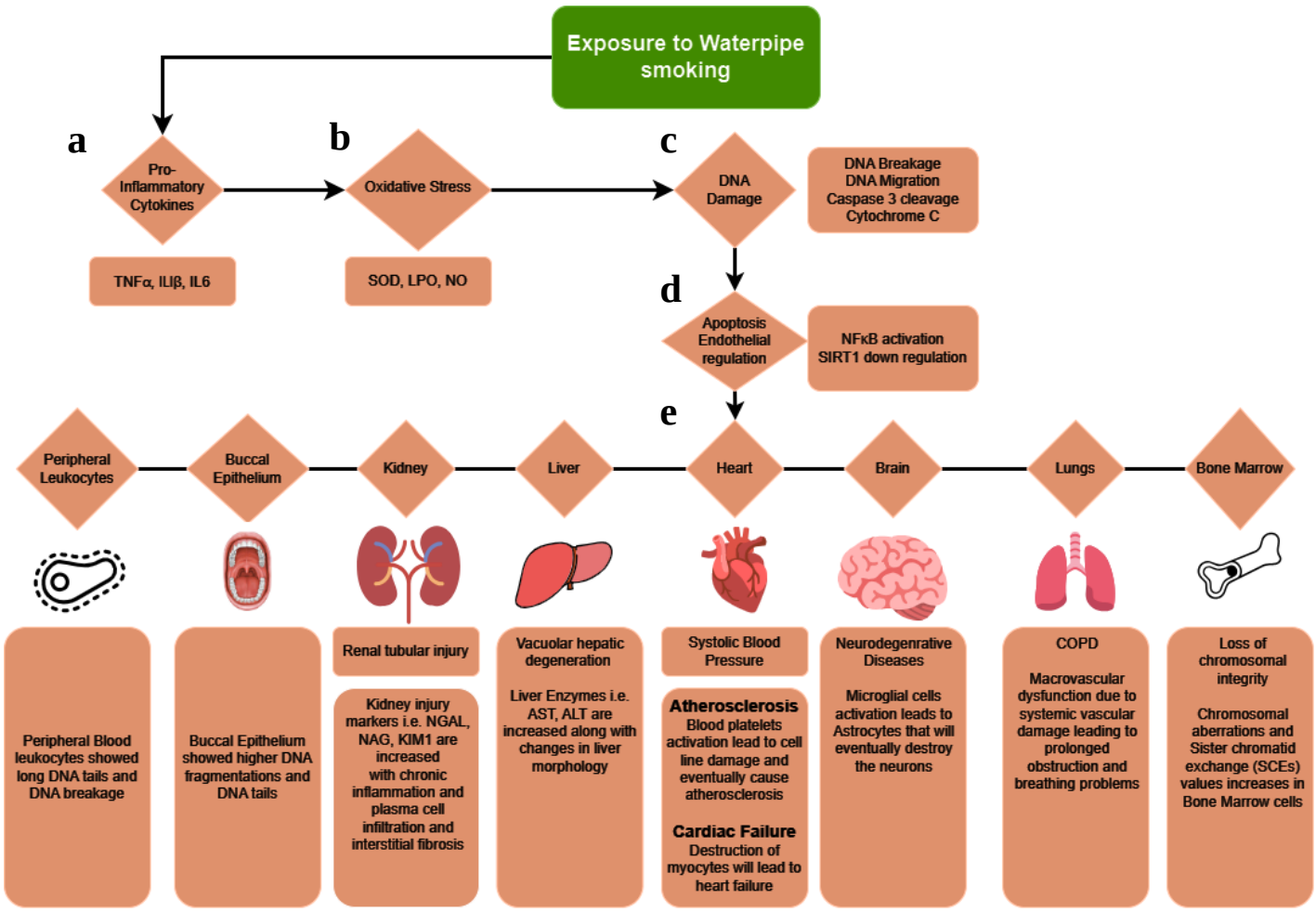

Fig. 4: Representation of possible chain of events in different organs upon exposure to waterpipe smoking (a) Pro-Inflammatory Cytokines: These are the chemicals that are released by immune cells to promote inflammation. These chemicals include TNF-α and interleukins such as IL-6, IL-1β, and IL-17. Their levels of concentration in the plasma can be measures using techniques such as ELISA. **(b) Oxidative stress:** It is the imbalance of production of ROS and the body's ability to detoxify them. There are some biomarkers being used to estimate the levels of oxidative stress such as Iso-prostanes, LPO, SOD, GSH, TBARS, CAT, 8-OHdG, GSSG etc. **(c) DNA damage:** Damage to DNA is a form of stress to cells and induce changes in genome which arises cancer and degenerative diseases. DNA damage includes DNA strand breakage, point mutations, chromosomal aberrations, micronucleus alterations, and reduction in DNA repair processes. There are various assays such as Comet Assay, Cytogenetic assays, Micronucleus assays, and Cell-cycle measurements techniques used to assess DNA damage. **(d) Cell death:** NF-κB activation leads to apoptotic cell death and down regulation of SIRT-1 gene responsible for endothelial regulation. Endothelial dysregulation leads to deterioration of endothelium of the organ. **(e) Organ-level degeneration:** Cell death and endothelial deterioration will eventually lead to effect the inner lining of organs which result in chronic diseases associated to organ. Common chronic and non-communicable diseases could be the result of exposure to waterpipe smoking. These life-long chronic diseases are problematic as well as fatal causing huge losses both in terms of economic burden and life.

But there are some limitations to this network analysis. For instance, this network does not capture all nuances, such as regional or demographic variations in waterpipe smoking behaviour. Future studies could benefit from incorporating these aspects for a more comprehensive understanding. This network analysis suggests that while considerable work has been done on cellular and animal models, more human-based studies may be required to confirm these findings in broader populations.

In conclusion, current study highlights the need of research studies focused on determining the effects of genotoxicity on humans using observational study designs. Furthermore, this study also indicates the need of understanding the pro-inflammatory cytokines which is often a first step after waterpipe smoking exposure. Understanding the genotoxicity in very early stages could help the humans in controlling the chronic diseases.

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions:

Muhammad Noman Ashraf: Conceptualization, Methodology, formal analysis. **Naghmana Aleem:** Conceptualization, Methodology. **Zabeehullah:** Methodology, formal analysis, Writing—original draft preparation. **Rida Junaid:** formal analysis, Writing—original draft preparation. **Aqeel Khan:** Conceptualization, Methodology, formal analysis, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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