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Antimutagenic potential of *Azadirachta indica* leaf extract in EMS: a qualitative study

Sasmita Das[1] and Durgapada Sarkhel[2]

[1]Department of Chemistry, Utkal University, Bhubaneswar, Odisha, India
[2]Department of Biotechnology, Utkal University, Bhubaneswar, Odisha, India

ABSTRACT

Background: Ethyl methanesulfonate (EMS) is a DNA-alkylating mutagen widely employed in experimental genotoxicity models. *Azadirachta indica* (neem) leaf extract contains bioactive phytochemicals with documented antioxidant and cytoprotective properties. However, a structured synthesis of expert perspectives regarding its antimutagenic relevance in EMS-induced genotoxicity remains limited.

Objective: This qualitative study aimed to synthesize multidisciplinary expert opinions on the biological plausibility, evidentiary strength, translational feasibility, and research gaps concerning the antimutagenic potential of A. indica leaf extract.

Methods: Semi-structured interviews were conducted with five professionals representing toxicology, phytochemistry, molecular biology, clinical pharmacology, and Ayurveda. Fourteen standardized questions explored EMS toxicity mechanisms, neem phytochemistry, methodological rigor, safety considerations, and future research directions. Responses were analyzed using thematic coding and stance classification.

Results: A total of seventy responses were analyzed. Most participants acknowledged the established mechanism of EMS-induced DNA alkylation and recognized neem's antioxidant properties as biologically plausible for mitigating oxidative stress. Supportive opinions predominated, though cautious and non-supportive perspectives highlighted methodological limitations, variability in extract standardization, insufficient EMS-specific studies, and absence of controlled clinical trials. Translational applicability was viewed as premature without further validation.

Conclusion: Expert perspectives indicate that A. indica leaf extract demonstrates mechanistic plausibility as a protective agent in experimental genotoxicity contexts. Nevertheless, methodological standardization, targeted EMS-specific studies, and clinical validation are required to establish its translational relevance.

KEY WORDS

Ethyl methanesulfonate; Azadirachta indica; Bioactive phytochemicals; Genotoxicity; Chemopreventive effects

ARTICLE HISTORY

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Introduction

Ethyl methanesulfonate (EMS) is a well-characterized monofunctional alkylating agent extensively used in genetic and toxicological research to induce point mutations. EMS exerts its mutagenic effect primarily through ethylation of DNA bases, particularly at the O^6 position of guanine, resulting in O^6-ethylguanine adduct formation [1]. This modification disrupts canonical base pairing during replication, frequently producing G:C to A:T transition mutations. Persistent DNA alkylation, if not repaired, causes genomic instability, chromosomal aberrations, and mutagenesis [2]. Due to its reproducibility and well-defined molecular action, EMS serves as a standard experimental model for evaluating genotoxicity and testing protective or antimutagenic agents. The cellular response to EMS exposure involves activation of DNA repair pathways, including direct reversal mechanisms mediated by O^6-alkylguanine-DNA alkyltransferase. However, incomplete repair or excessive alkylation may result in mutation fixation and long-term genetic consequences [3].

Natural products and plant-derived phytochemicals have gained increasing attention as potential modulators for oxidative stress and genotoxic injury. Several medicinal plants have demonstrated antigenotoxic and cytoprotective properties in experimental systems, often attributed to antioxidant and anti-inflammatory mechanisms [4]. Among these, *Azadirachta indica* (neem) occupies a prominent position due to its extensive traditional use and growing pharmacological validation [5]. Neem leaves contain diverse bioactive constituents, including flavonoids, polyphenols, triterpenoids, and limonoids such as nimbin and azadirachtin. Experimental studies have reported antioxidant, anti-inflammatory, antimicrobial, and chemopreventive effects of neem extracts in various vitro and in vivo models [6].

EMS-induced DNA damage involves both direct alkylation and secondary oxidative stress pathways. Given that oxidative stress contributes to amplification of DNA lesions, compounds with antioxidant properties may theoretically reduce mutation burden or cellular injury following EMS exposure. Neem leaf extract, with documented radical-scavenging activity, presents a plausible candidate for such protective effects [7].

The present study adopts a qualitative research design based on semi-structured expert interviews. Professionals from diverse but relevant disciplines were selected to provide multidisciplinary perspectives. A standardized interview guide was developed to explore mechanisms of EMS toxicity, phytochemical properties of neem, strength of evidence for antimutagenic activity, translational feasibility, and safety

***Correspondence:** Ms. Sasmita Das, Department of Chemistry, Utkal University, Odisha, India, e-mail: sasmitadas542@gmail.com

considerations. Data were analyzed using thematic coding to identify recurrent patterns, convergences, and divergences across professional viewpoints.

A major limitation in the current body of literature is the absence of an integrated evaluation that combines mechanistic toxicology, phytochemical characterization, translational assessment, and safety appraisal within a single analytical framework. Most published studies focus either on experimental outcomes without standardized extract profiling or on traditional medicinal claims without molecular validation. Furthermore, there is limited systematic assessment of expert consensus regarding the plausibility and applicability of neem as an antimutagen in EMS models. The objective of this qualitative study is to systematically synthesize multidisciplinary expert perspectives to evaluate the biological plausibility, evidentiary strength, translational feasibility, and research gaps concerning the antimutagenic potential of *Azadirachta indica* leaf extract against EMC-induced genotoxicity.

Research Questions

- How do experts assess the current experimental evidence supporting neem's antimutagenic potential?
- What methodological and standardization challenges limit interpretation of existing findings?
- Is there plausible involvement in antioxidant or DNA repair modulation pathways?
- What are the translational, safety, and regulatory considerations associated with potential therapeutic applications?
- What future research directions are necessary to strengthen the evidence base?

Methodology

This study was designed as a qualitative investigation aimed at synthesizing multidisciplinary expert perspectives regarding the antimutagenic potential of *Azadirachta indica* leaf extract in the context of EMS-induced genotoxicity. A semi-structured interview methodology was used to obtain in-depth analytical insights across relevant scientific and clinical domains. The study followed a thematic analysis framework to identify patterns, convergence, and divergence of expert opinion.

Inclusion criteria required

- A minimum of five years of professional or research experience in the relevant discipline.
- Familiarity with genotoxicity, oxidative stress mechanisms, phytochemical research, or translational medicine.

Exclusion criteria included

- Absence of formal disciplinary training.
- No documented experience in chemical mutagenesis, phytomedicine research, or clinical pharmacology.

Five professional categories were included:

- Toxicologist
- Phytochemist
- Molecular Biologist
- Clinical Pharmacologist
- Ayurveda Practitioner

A semi-structured interview guide was developed following review of published literature on EMS-induced genotoxicity and plant-derived antimutagenic compounds. Interviews were conducted individually through in-person or virtual platforms, depending on participant availability. Each interview lasted approximately 30-45 minutes. Clarification probes were used where necessary to ensure conceptual accuracy. Audio recordings were obtained with participant consent (Table 1).

The study was conducted in accordance with accepted ethical standards for qualitative research involving human participants. Participants were informed of the study purpose, confidentiality measures, voluntary participation, and intended academic dissemination. Written or recorded verbal informed consent was obtained prior to data collection.

A coding framework was established based on study objectives and literature review. Eight primary codes were defined:

- T1: Mechanism of EMS-induced DNA damage
- T2: Phytochemical and antioxidant properties of neem
- T3: Evidence strength and methodological limitations
- T4: DNA repair modulation
- T5: Translational feasibility
- T6: Safety and regulatory considerations
- T7: Role of traditional medicine
- T8: Preventive healthcare implications

Table 1 . Table representing the interview of the participants in the qualitative research model.

Theme Code	Question	Participants	Response	Opinion
T1	How would you describe the mechanism of toxicity induced by Ethyl Methanesulfonate (EMS)?	Toxicologist	EMS directly alkylates DNA, especially guanine bases, which leads to mutations during replication.	Supportive
		Phytochemist	It chemically modifies DNA bases, which disrupts normal genetic stability.	Neutral
		Molecular Biologist	It forms O6-ethylguanine, causing incorrect base pairing and point mutations.	Supportive
		Clinical Pharmacologist	It induces genetic instability through DNA alkylation.	Neutral
		Ayurveda Practitioner	It appears to damage genetic material at a molecular level.	Supportive

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T1	What types of cellular or DNA damage are most associated with EMS exposure?	Toxicologist	We typically see point mutations and chromosomal aberrations.	Supportive
		Phytochemist	There's oxidative stress and base mispairing involved.	Neutral
		Molecular Biologist	Mainly single-base substitutions during replication.	Supportive
		Clinical Pharmacologist	Genotoxic stress that may trigger apoptosis.	Neutral
		Ayurveda Practitioner	It causes cellular imbalance and structural damage.	Neutral
T2	What are your understanding of the medicinal properties of *Azadirachta indica* (Neem)?	Toxicologist	Neem has well-documented antioxidant and anti-inflammatory properties.	Supportive
		Phytochemist	It's rich in flavonoids and limonoids with biological activity.	Supportive
		Molecular Biologist	Some studies show cytoprotective effects.	Cautious
		Clinical Pharmacologist	It has traditional use, but strong clinical validation is limited.	Non-Supportive
		Ayurveda Practitioner	Neem is traditionally used for detoxification and immune support.	Supportive
T2	Which bioactive components in neem leaves do you believe may contribute to protective effects?	Toxicologist	Flavonoids likely contribute to the antioxidant effect.	Supportive
		Phytochemist	Quercetin, nimbin, and azadirachtin are key compounds.	Supportive
		Molecular Biologist	Compounds that scavenge ROS are most relevant.	Supportive
		Clinical Pharmacologist	Polyphenols may play a role, but dosing is unclear.	Cautious
		Ayurveda Practitioner	The bitter phytochemicals are considered protective.	Supportive
T2	Explain a protective effect of neem against DNA damage?	Toxicologist	It likely reduces oxidative stress before DNA damage occurs.	Supportive
		Phytochemist	It neutralizes reactive oxygen species.	Supportive
		Molecular Biologist	It may limit secondary oxidative damage.	Cautious
			The mechanism isn't clearly proven.	Non-Supportive
		Ayurveda Practitioner	It strengthens the body's defense system.	Supportive
T3	Based on available studies, how strong do you consider the evidence supporting neem's protective role against genotoxic agents?	Toxicologist	There's moderate support from preclinical models.	Cautious
		Phytochemist	Lab data are fairly consistent.	Supportive
		Molecular Biologist	Large-scale validation is still lacking.	Cautious
		Clinical Pharmacologist	Clinical data are insufficient.	Non-Supportive
		Ayurveda Practitioner	There is strong traditional backing.	Cautious
T3	Do you think neem leaf extract has scientifically antimutagenic properties? Why or why not?	Toxicologist	Animal studies suggest it may reduce mutation rates.	Supportive
		Phytochemist	Preclinical evidence supports antimutagenic potential.	Supportive
		Molecular Biologist	It's plausible, but more molecular validation is needed.	Cautious
		Clinical Pharmacologist	There's not enough clinical evidence yet.	Non-Supportive
		Ayurveda Practitioner	Traditional knowledge supports its protective role.	Supportive
		Toxicologist	Sample sizes are often small.	Critical

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		Phytochemist	Extract standardization is inconsistent.	Critical
T3	Are there methodological limitations in current studies evaluating neem's antimutagenic potential?	Molecular Biologist	Mechanistic depth is limited.	Critical
		Clinical Pharmacologist	There are no controlled human trials.	Critical
		Ayurveda Practitioner	Traditional practices are not always quantified.	Cautious
		Toxicologist	It's possible, but not clearly demonstrated.	Cautious
T4	Is there any possibility that neem could modulate DNA repair mechanisms?	Phytochemist	Some indirect effects are suggested.	Cautious
		Molecular Biologist	Direct evidence is weak.	Non-Supportive
		Clinical Pharmacologist	There's no strong proof yet.	Non-Supportive
		Ayurveda Practitioner	It is traditionally believed to restore balance.	Supportive
		Toxicologist	It needs more research before translation.	Cautious
T5	From your professional perspective, how realistic is the use of these findings into human health applications?	Phytochemist	It could be feasible with proper standardization.	Supportive
		Molecular Biologist	It's too early to conclude.	Cautious
		Clinical Pharmacologist	It's not ready for clinical use.	Non-Supportive
		Ayurveda Practitioner	It's already used traditionally.	Supportive
		Toxicologist	High doses may pose toxicity risks.	Cautious
T6	What safety concerns should be considered when using neem extract therapeutically?	Phytochemist	Purity and dose control are essential.	Cautious
		Molecular Biologist	Long-term genomic effects are unclear.	Cautious
		Clinical Pharmacologist	There may be herb–drug interactions.	Cautious
		Ayurveda Practitioner	It is safe when properly administered.	Supportive
		Toxicologist	They're valuable sources of bioactive compounds.	Supportive
T7	How do you view the role of traditional medicinal plants in modern toxicology research?	Phytochemist	They're important for drug discovery.	Supportive
		Molecular Biologist	They're useful if mechanistically validated.	Cautious
		Clinical Pharmacologist	They must meet evidence standards.	Cautious
		Ayurveda Practitioner	They are central to preventive healthcare.	Supportive
		Toxicologist	Larger controlled animal studies.	Supportive
T8	What type of future research would you recommend strengthening the evidence base?	Phytochemist	Standardized extract profiling.	Supportive
		Molecular Biologist	Molecular pathway analysis.	Supportive
		Clinical Pharmacologist	Well-designed human trials.	Supportive
		Ayurveda Practitioner	Integrative traditional-modern research.	Supportive

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T8	In your opinion, can plant-based antimutagens play a significant role in preventive healthcare?	Toxicologist	Possibly, if evidence strengthens.	Cautious
		Phytochemist	They can serve as adjunct preventive agents.	Supportive
		Molecular Biologist	Only after strong validation.	Cautious
		Clinical Pharmacologist	Not without clinical proof.	Non-Supportive
		Ayurveda Practitioner	They have strong preventive potential.	Supportive

Thematic analysis was performed through iterative review of transcripts to identify recurring conceptual patterns and disciplinary differences. Codes were aggregated into clusters corresponding to the framework. Comparative analysis was conducted across professional categories to assess areas of consensus and divergence.

Results

Each participant responded to fourteen standardized questions, generating a total of seventy responses. All participants had domain-specific expertise relevant to toxicology, phytochemistry, molecular biology, pharmacology, or traditional medicine systems. The inclusion of multidisciplinary perspectives enabled comparison across mechanistic, chemical, translational, and traditional viewpoints (Table 2).

Table 2. Response analysis of the participants.

Response	Count	Percentage
Supportive	30	47.60%
Neutral	5	7.90%
Cautious	16	25.40%
Non-Supportive	30	12.70%
Critical	4	6.30%

Participants under T1 demonstrated high mechanistic consistency. Toxicologist and Molecular Biologist responses were strongly supportive of established alkylation pathways, emphasizing O^6-ethylguanine formation and point mutations. The Phytochemist and Clinical Pharmacologist provided neutral interpretations consistent with DNA base modification and genetic instability. The Ayurveda Practitioner described cellular-level genetic damage without mechanistic detail.

Theme 2 demonstrated predominantly supportive perspectives. Out of fifteen responses under T2, eleven were classified as supportive. Toxicologist and Phytochemist responses highlighted flavonoids, limonoids, and antioxidant activity as mechanistically relevant. The Molecular Biologist adopted a cautious tone regarding cytoprotective claims. The Clinical Pharmacologist expressed a non-supportive stance in relation to clinical validation, while remaining cautious regarding mechanistic certainty. The Ayurveda Practitioner consistently supported traditional detoxification and immune-modulatory roles.

Theme 3 demonstrated the highest variability. Responses addressing evidence strength showed divergence among disciplines. While the Phytochemist and Toxicologist described preclinical support, the Molecular Biologist and Clinical Pharmacologist emphasized insufficient large-scale validation and limited clinical data. This theme generated the highest concentration of critical and non-supportive responses, indicating the concerns about methodological practice represent a major area of consensus across disciplines.

Responses under T4 were predominantly cautious or non-supportive. The Toxicologist and Phytochemist acknowledged possible indirect effects but emphasized limited demonstration. The Molecular Biologist and Clinical Pharmacologist explicitly stated that direct evidence for DNA repair modulation is weak or absent. The Ayurveda Practitioner provided a supportive response grounded in traditional belief rather than molecular data.

Under T5 the Phytochemist and Ayurveda Practitioner expressed supportive positions. The Toxicologist and Molecular Biologist were cautious, citing the need for further validation. The Clinical Pharmacologist adopted a non-supportive stance, indicating insufficient readiness for clinical use.

Under T6, four of five participants expressed cautious opinions. Toxicologist, Phytochemist, Molecular Biologist, and Clinical Pharmacologist identified potential concerns including toxicity at high doses, purity control, long-term genomic safety, and herb–drug interactions. Only the Ayurveda Practitioner expressed supportive opinion, conditional upon proper administration.

Theme 7 generated supportive responses from Toxicologist, Phytochemist, and Ayurveda Practitioner, emphasizing the value of traditional plants as bioactive sources and preventive tools. Molecular Biologist and Clinical Pharmacologist adopted cautious positions, stressing the need for validation and adherence to evidence standards.

Future research recommendations were uniformly supportive across all participants, indicating on the need for controlled animal studies, molecular pathway analysis, extract standardization, and human trials.

The Ayurveda Practitioner and Phytochemist demonstrated the highest percentage of supportive responses. The Toxicologist showed a mixed but largely supportive approach with methodological caution. The Molecular Biologist exhibited a predominantly cautious approach with limited supportive responses. The Clinical Pharmacologist demonstrated the highest proportion of non-supportive responses, particularly regarding clinical translation and DNA repair claims (Table 3 and Figure 1).

Table 3. Number of responses from the participants.

Participant	Supportive	Neutral	Cautious	Non-Supportive	Critical
Toxicologist	8	0	5	0	1
Phytochemist	9	2	2	0	1

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Molecular Biologist	4	0	8	1	1
Clinical Pharmacologist	1	2	3	7	1
Ayurveda Practitioner	11	1	2	0	0

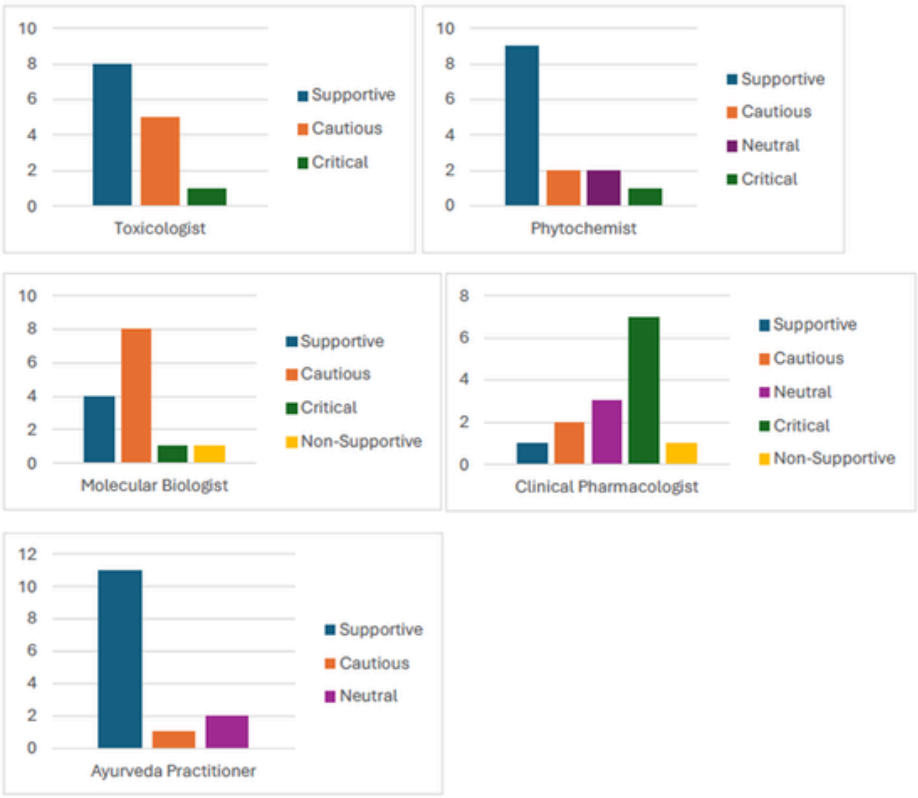

Figure 1. Graphs representing different responses from each participant.

Discussion

The present qualitative findings should be interpreted within the context of existing experimental and toxicological research on *Azadirachta indica* and chemically induced genotoxicity models. EMS is a well-characterized monofunctional alkylating agent that primarily induces O^6-ethylguanine formation, resulting in GC→AT transition mutations during DNA replication [8]. EMS has been extensively used in mutagenesis and genotoxicity testing because of its predictable DNA-alkylating mechanism and reproducible induction of point mutations. Its mutational profile provides a suitable experimental platform to evaluate protective agents with antioxidant or DNA-protective properties [9].

Reviews have documented that *Azadirachta indica* contains diverse bioactive constituents. These compounds are widely recognized for antioxidant and free radical-scavenging activity. Experimental data suggest that neem leaf extracts enhance endogenous antioxidant enzymes such as superoxide dismutase and catalase, reduce lipid peroxidation, and modulate redox-sensitive signaling pathways [10]. Several in vivo and in vitro investigations have evaluated neem in carcinogenesis and mutagenesis models. The Allium cepa assay and Ames test have been used to evaluate cytotoxicity and chromosomal alterations associated with neem extracts [11]. In these studies, low to moderate concentrations of neem leaf extracts demonstrated protective or non-mutagenic profiles, whereas higher concentrations were associated with chromosomal bridges, c-mitosis, or mitotic index alterations. Such dose-dependent dual effects highlights the importance of concentration standardization and toxicological threshold definition [12,13] (Table 4).

Table 4. Phytochemtical components in the Neem leaf extract.

Bioactive Contents	Extract
Saponins	Marked
Tannin	Present
Terpens	Absent
Anthraquinone	Absent
Deoxysugars	Absent
Steroids	Absent
Flavonoids	Present
Alkaloids	Present
Phenolic	Present

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Additional investigations have explored antimicrobial, anti-inflammatory, antihyperglycemic, and immunomodulatory activities of neem leaf extracts. The antimutagenic and anticarcinogenic properties described in these reviews are frequently attributed to antioxidant-mediated suppression of reactive oxygen species and modulation of cellular signaling pathways involved in apoptosis and proliferation [14]. However, while preclinical data are substantial, most studies remain confined to experimental models. While epidemiological and traditional data suggest broad usage, well-designed randomized controlled trials evaluating genomic stability endpoints or mutagenic biomarkers in humans are lacking. Current clinical evidence primarily addresses metabolic disorders, inflammatory conditions, or adjunctive oncological support rather than direct antimutagenic assessment [15].

From a scientific perspective, the evidence supports continued investigation under controlled experimental conditions. Future work should prioritize standardized phytochemical quantification, EMS-specific mechanistic assays, genomic damage biomarkers, dose-response characterization and controlled human studies.

Conclusion

This qualitative investigation evaluated interdisciplinary expert perspectives on the antimutagenic potential of *Azadirachta indica* leaf extract in relation to EMS-induced genotoxicity. The findings demonstrate broad agreement regarding the established DNA-alkylating mechanism of EMS and its reliability as an experimental mutagen. Neem leaf extract was consistently recognized as a biologically active phytotherapeutic agent with antioxidant and cytoprotective properties supported by preclinical evidence. The presence of flavonoids, limonoids, and other polyphenolic constituents provides a mechanistic basis for reduction of oxidative stress and mitigation of secondary DNA damage. The analysis identified significant limitations in the current evidence base. Most available data derive from in vitro and animal models, with limited EMS-specific investigations. Variability in extraction methods, incomplete phytochemical standardization, and inconsistent characterization restrict reproducibility across studies. Experimental genotoxicity assessments indicate that biological effects may be dose-dependent, highlighting the need to define therapeutic thresholds. Furthermore, there is a lack of controlled human studies evaluating genomic stability biomarkers, which limits translational interpretation. Overall, the findings suggest that neem leaf extract demonstrates potential protective effects under experimental conditions but cannot yet be considered a clinically validated antimutagenic intervention. The divergence in professional perspectives reflects the balance between biochemical plausibility and translational insufficiency. Future research should focus on EMS-specific mechanistic studies examining DNA adduct formation, repair modulation, and oxidative stress biomarkers. Standardized extraction protocols with detailed phytochemical profiling are essential for reproducibility. Controlled in vivo studies should establish safety margins and dose response relationships. Well-designed clinical trials assessing pharmacokinetics, safety, and genomic endpoints are necessary to determine therapeutic applicability. In conclusion, *Azadirachta indica* leaf extract represents a promising but methodologically underdeveloped candidate for mitigating chemically induced genotoxic stress. Advancement requires rigorous experimental validation and clinical evaluation.

Disclosure Statement

The authors do not have any conflict of interest.

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