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CYTOTOXICITY OF NEEM (*Azadirachta indica*) SEED OIL AND ITS RELEVANCE FOR INHALATION EXPOSURE

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ABSTRACT

Widely perceived as a natural and safer alternative to synthetic pesticides, neem (*Azadirachta indica*) seed oil is extensively used as a biopesticide in agriculture; however, its potential effects following inhalation exposure remain insufficiently characterized. This study assessed its cytotoxicity in the human lung epithelial A549 cell line using the MTT assay. A significant concentration-dependent decrease in cell viability was observed, with minimal effects at low concentrations and pronounced cytotoxicity at higher concentrations. These findings indicate that neem seed oil can exert cytotoxic effects relevant to inhalation exposure, emphasizing the need for careful safety evaluation under realistic conditions of use. Further studies are warranted to better characterize the mechanisms of toxicity and to support a more comprehensive evaluation of the safety of neem-based biopesticides.

INTRODUCTION

To date, thousands of plant species have been evaluated for pest and disease control in modern agriculture, leading to the widespread use of numerous horticultural mineral oils, botanical extracts, and essential oil-based formulations. Among these, neem (*Azadirachta indica*) seed oil has gained widespread use owing to its reported insecticidal, antifungal, and antibacterial effects, which are attributed to its complex chemical composition, rich in bioactive compounds such as azadirachtin, nimbin, salannin, and various fatty acids [1].

While neem oil is often considered relatively safe based on traditional use and low acute toxicity in mammals, its complex chemical composition and formulation additives may trigger adverse cellular responses under certain exposure conditions [2]. Neem-based formulations are commonly applied as sprays, generating aerosols and fine droplets that can be inhaled by operators and individuals in the surrounding environment. In this context, its increasing use as

a sprayable biopesticide has raised questions about human exposure under realistic use conditions. This study aims to investigate its cytotoxic potential using the human lung epithelial cell line A549 as a model system. The A549 cell line is commonly used in toxicological research as a model for respiratory exposure and to evaluate the cytotoxic effects of inhaled toxicants [3]. By focusing on inhalation exposure scenarios, the study's findings are intended to contribute to a more comprehensive understanding of the safety profile of neem-based biopesticides and to support their responsible use.

EXPERIMENTAL

Preparation of neem seed oil treatment solutions

Commercially available, cold-pressed neem (*Azadirachta indica*) seed oil (Eterra, TemaCo d.o.o., Novi Sad, Serbia; country of origin: India) was used in the experiments. Stock emulsions of neem oil were prepared by mixing the oil with the surfactant Tween 20 (Sigma-Aldrich, France) and Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, USA). Working concentrations were prepared by serial dilution.

Cell culture

A549 cells (ATCC CCL-185, Manassas, VA, USA) were cultured in DMEM supplemented with 10% fetal bovine serum (Capricorn Scientific, Germany) and 1% penicillin/streptomycin (Capricorn Scientific, Germany) at 37 °C in 5% CO_2. Cell viability and density were assessed by trypan blue exclusion, as previously described [4]. Cells were seeded in 96-well plates (1×10^4 cells/well), allowed to attach and form a monolayer for 24 h, and then treated with neem oil at final concentrations of 0.01–2 mg/mL for 24 h. Untreated cells served as negative controls. The final concentration of Tween 20 in the cultures did not exceed the toxicity threshold (<0.01%) [5], except at 2 mg/mL neem seed oil, where it reached 0.02%, so a corresponding solvent control (0.02% Tween 20) was included. All experiments were performed in triplicate.

Cytotoxicity assessment (MTT assay)

Cytotoxicity was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The absorbance was measured at 570 nm (reference 630 nm). Cell viability was expressed relative to untreated controls. The IC_{50} was defined as the concentration that reduced cell viability by 50%.

Statistical analysis

Statistical analysis was performed using the Student's t-test with Statistica 8 and OriginPro 8.5.1 for Microsoft Windows. The p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results of the study demonstrate a concentration-dependent cytotoxic effect of neem oil after 24 h of exposure, with substantial reductions in cell viability at concentrations ≥0.5 mg/mL ($p < 0.05$) and near-complete loss of viability at 2

mg/mL ($p < 0.001$) (Fig. 1.). The calculated IC_{50} value (0.89 mg/mL) indicates moderate cytotoxic potency under the tested conditions. While cell viability remained above ~85–90% at concentrations ≤0.025 mg/mL, suggesting limited acute toxicity in this range, the progressive decline observed at 0.05 mg/mL and above indicates the onset of cellular stress.

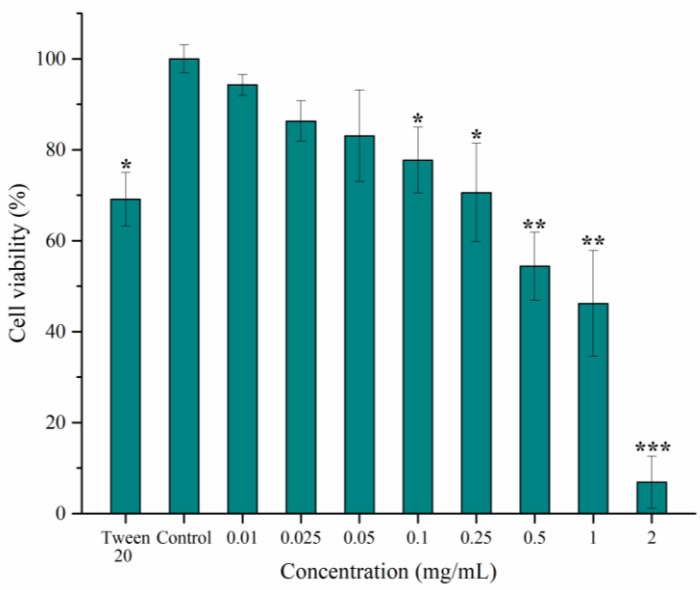


Figure 1. Cell viability (%) (mean ± SD) following exposure to increasing concentrations of neem seed oil, calculated relative to the untreated control (100%) for all tested concentrations. *p<0.05, **p<0.01, ***p<0.001 compared to control.

At the highest concentration, viability decreased to 6.88%, confirming pronounced cytotoxicity. Notably, treatment with Tween 20 (0.02%) alone resulted in approximately 70% viability, indicating that the additional reduction is primarily attributable to the intrinsic toxicity of neem oil rather than the surfactant. This distinction is important, as it highlights that the biological activity of neem-based formulations may not always be attributed solely to the active substance but may instead reflect the combined, potentially additive effects of both active and formulation constituents. The observed cytotoxicity of neem oil may be associated with mitochondrial dysfunction and disruption of membrane integrity, leading to impaired metabolic activity. Limonoids such as azadirachtin are known to interfere with multiple cellular signaling pathways and may contribute to the observed effects [6].

From a safety perspective, concentrations that induce pronounced cytotoxicity exceed those typically encountered in environmental exposure; however, localized exposure, such as inhalation of aerosolized droplets during spraying or

occupational handling, may transiently elevate concentrations at the epithelial surface. A recent study further supports this concern, showing a significantly higher prevalence of respiratory symptoms and impaired lung function in workers exposed to neem oil processing compared with controls, indicating adverse effects from aerosols, dust, and associated chemicals [7].

CONCLUSION

The observed cytotoxicity in A549 cells suggests that inhaled neem oil may adversely affect the respiratory epithelium. Further studies are warranted to better characterize its safety profile under realistic exposure conditions.

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