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RESEARCH ARTICLE

AN UPGRADATION TO MINIMIZE HAZARDOUS EFFECTS IN HISTOPATHOLOGICAL LABORATORIES: CEDARWOOD OIL AS AN ALTERNATIVE TO XYLENE

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Abstract

Background: In traditional histopathology, xylene is used as a clearing agent following haematoxylin and eosin (H&E) staining of tissue sections. However, xylene presents significant health risks, prompting the search for safer alternatives. Essential oils, including cedarwood oil, have been proposed as potential replacements. This study evaluates the efficacy of cedarwood oil as a clearing agent compared to xylene.

Aim: To assess the clearing effectiveness of cedarwood oil as an alternative to xylene in routine haematoxylin and eosin staining.

Materials and Methods: Twenty paraffin blocks from routine biopsy specimens were used. Cedarwood oil, sourced from a local organic supplier, was tested alongside xylene. Paraffin sections (4 microns) from each block were cleared using either 8% cedarwood oil or xylene and subsequently stained with H&E. The quality of staining was evaluated based on nuclear and cytoplasmic detail, clarity, and uniformity.

Results: Cedarwood oil demonstrated a significant correlation with xylene concerning all assessed staining parameters.

Conclusion: Cedarwood oil is a viable, eco-friendly, and safer alternative to xylene for use as a clearing agent in histopathological laboratories.

Keywords: *Cedarwood oil, clearing agent, histopathology, xylene, tissue processing, SDG3.*

INTRODUCTION

Clearing is a crucial step in the preparation of histopathological tissue for light microscopy, aimed at removing dehydrating agents from previous processing stages and preparing tissues for embedding.[1] Xylene has long been the gold standard for this purpose.[2] However, inhalation of xylene vapours can lead to central nervous system depression symptoms such as headaches, dizziness, nausea, and vomiting. Prolonged exposure can result in anxiety, insomnia, agitation, chronic fatigue, tremors, impaired concentration, and memory issues. Xylene, a toxic and carcinogenic aromatic hydrocarbon, poses significant long-term health risks to technicians and pathologists.[3] Alternatives to xylene include limonene reagents, aliphatic hydrocarbon mixtures, mineral oils, and aromatic hydrocarbon mixtures with lower volatility. [4] Among these, cedarwood oil stands out as a viable substitute.

Cedarwood oil offers a safe, natural, and eco-friendly alternative to xylene. It is non-flammable, readily available, easy to handle, and importantly, non-carcinogenic.[5] Additionally, cedarwood oil possesses antiseptic, antifungal, antibacterial, and antispasmodic properties. It also has effective clearing capabilities, acting gently on tissues without causing damage.[6] Its health benefits outweigh its cost, making it suitable as a deparaffinization agent in H&E staining procedures.[7] These attributes make cedarwood oil a compelling option compared to traditional clearing agents in histopathological laboratories. The present study aimed to evaluate effectiveness of cedarwood oil as a clearing agent in routine tissue processing, comparing it to xylene.

MATERIALS AND METHODS

The study utilized tissue sections from the archives of the Department of Oral Pathology and Microbiology, with institutional ethical approval number KSDEC/23-24/Apr/018A. It involved 40 tissue samples, split into two groups. Group A comprised 20 tissue sections cleared with cedarwood oil (Isturon Chemicals, refractive index at 25°C = 1.49-1.525, weight per ml at 25°C = 1.01-1.22g) as an alternative to xylene, while Group B included 20 specimens cleared with routine xylene (Merck Life Science Pvt. Ltd, M = 106.17g/mol, density = 20°C). The cedarwood oil solution was made by mixing 5 ml of xylene with 95 ml of cedarwood oil to prevent crystalline cedrol formation, with a few thymol crystals added for disinfection. Sections in Group A were cleared using this cedarwood oil solution, while Group B sections were cleared with xylene. Staining was performed using the routine haematoxylin and eosin method (Figure 1).

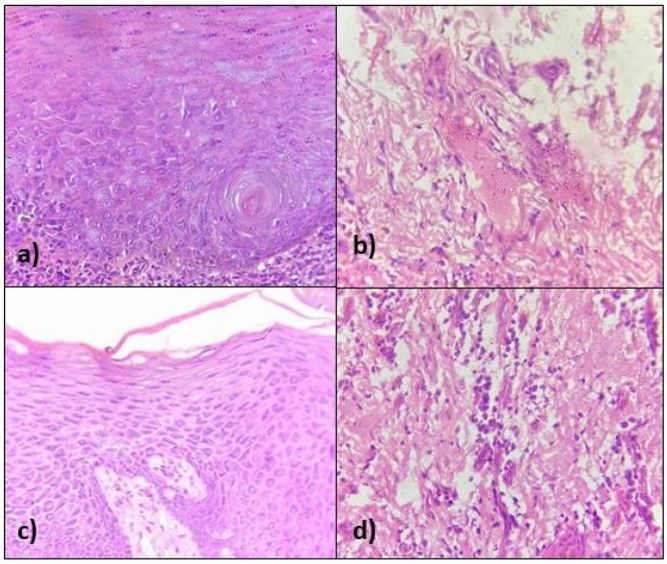

Figure 1. *Photomicrographs showing comparison of haematoxylin and eosin staining quality obtained by using xylene as a clearing agent. a) Photomicrographs (H and E stain, 40X magnification) showing* ***a)*** *Epithelium of tissue section prepared by using cedarwood oil solution as a clearing agent.* ***b)*** *Connective tissue of tissue section prepared by using cedarwood oil solution as a clearing agent.* ***c)*** *Epithelium of tissue section prepared by using cedarwood oil solution as a clearing agent.* ***d)*** *Connective tissue of tissue section prepared by using cedarwood oil solution as a clearing agent*

Scoring criteria used was as follows: Nuclear and cytoplasmic staining: (0) Poor Staining with poor morphology, (1) fair staining, (2) good staining. Artifacts staining: (0) Nil, (1) mild, (2) moderate. The scores of samples processed by the clearing method between cedarwood oil solution and xylene were tabulated and compared with Chi Square Test of Proportion and Mann Whitney U Test. All the statistical analysis were performed using IBM Statistical Package for Social Science version 21. The tests were performed keeping confidence interval at 95% and ($p<0.05$) was considered to be statistically significant.

RESULTS

The study compared tissue samples processed with xylene and cedarwood oil by examining percentage values for various parameters, including background staining, nuclear staining, cytoplasmic staining, and artifacts.

Twenty tissue samples processed with cedarwood oil were categorized into two subgroups: A) epithelium and B) connective tissue. The results for epithelial tissues showed good staining in 23% of the samples, fair staining in 60%, and poor staining in 17%. The chi-square test value for these samples was 8.65 with a p-value of 0.7. For connective tissue processed with cedarwood oil, the outcomes were similar to those for epithelium. Nuclear staining yielded fair results in 65% of samples, with cytoplasmic staining and artifacts each showing fair results in 45% of samples. The percentages for good staining were 25% for nuclear staining, 45% for cytoplasmic staining, and 40% for artifacts. The proportion of poor staining was minimal. The chi-square test value was 2.49 with a p-value of 0.64 (Table 1).

Table 1: *Summary of haematoxylin and eosin staining quality obtained by using cedarwood oil solution as a clearing agent*

Part of the tissue section		Parameters				Statistics	
		Good	Fair	Poor	Total	Chi value	P value
Epithelium	Nucleus	6 (30%)	12 (60%)	2 (10%)	20	8.65	0.7
	Cytoplasm	6 (30%)	13 (65%)	1 (5%)	20		
	Artifacts	2 (10%)	11 (55%)	7 (35%)	20		
	Total	14 (23%)	36 (60%)	10 (17%)	60		
Connective tissue	Nucleus	5 (25%)	13 (65%)	2 (10%)	20	2.49	0.64
	Cytoplasm	9 (45%)	9 (45%)	2 (10%)	20		
	Artifacts	8 (40%)	9 (45%)	3 (15%)	20		
	Total	22 (37%)	31 (52%)	7 (11%)	60		

Twenty tissue samples treated with xylene were also evaluated, divided into two categories: A) epithelium and B) connective tissue, focusing on nuclear staining, cytoplasmic staining, and artifacts. For epithelial tissues, xylene processing resulted in good staining in 18% of samples, fair staining in 68%, and poor staining in 14%. The chi-square test value was 9.20 with a p-value of 0.056. For connective tissues processed with xylene, the results were comparable to those for epithelial tissues. Fair staining for nuclear staining was observed in 60% of samples, with cytoplasmic staining at 55% and artifacts at 80%. The percentages for good staining were 35% for artifacts, 35% for cytoplasmic staining, and 10% for nuclear staining, with the poor category showing the least percentage. The chi square value was 4.60 with a p-value of 0.3 (Table 2).

Table 2: *Summary of haematoxylin and eosin staining quality obtained by using xylene as a clearing agent*

Part of the tissue section		Parameters				Statistics	
		Good	Fair	Poor	Total	Chi value	P value
Epithelium	Nucleus	5 (25%)	14 (70%)	1 (5%)	20	9.2	0.056
	Cytoplasm	5 (25%)	14 (70%)	1 (5%)	20		
	Artifacts	1 (5%)	13 (65%)	6 (30%)	20		
	Total	11 (18%)	41 (68%)	8 (14%)	60		
Connective tissue	Nucleus	7 (35%)	12 (60%)	1 (5%)	20	4.6	0.33
	Cytoplasm	7 (35%)	11 (55%)	2 (10%)	20		
	Artifacts	2 (10%)	16 (80%)	2 (10%)	20		
	Total	16 (27%)	39 (65%)	5 (8%)	60		

The comparison of the results from both groups indicated nearly similar outcomes. The p-value of 0.22, suggesting no significant difference in cytoplasmic and nuclear staining between the two clearing agents. However, a significant difference was noted in the presence of artifacts, which were more prevalent in samples processed with xylene (Table 3).

Table 3: *Comparison of Cedarwood oil solution and Xylene as a clearing agent in routine haematoxylin and eosin staining of tissue sections*

Group	Good	Fair	Poor	Total	P Value
Group A	36 (30%)	67 (56%)	17 (14%)	120	0.22
Group B	27 (22%)	80 (67%)	13 (11%)	120	

DISCUSSION

Since the 1950s, xylene has been the predominant clearing agent used in histopathology laboratories, establishing itself as the industry standard. However, its use is accompanied by significant health and environmental concerns. Xylene vapours, being denser than air, tend to accumulate and are rapidly absorbed through inhalation or ingestion. Exposure levels exceeding 200 ppm can lead to systemic toxicity, with an acceptable limit set at 100 ppm. Higher concentrations can cause irritation to the skin, eyes, and respiratory system, with an odour threshold of approximately 1 ppm.[7,8] Additionally, xylene's disposal poses further

challenges due to its toxicity.[9]

Given these issues, the present study aimed to identify a safer alternative. Among the various substitutes, such as limonene reagents, aliphatic hydrocarbons, and mineral oils, cedarwood oil emerged as a promising candidate. Cedarwood oil is non-carcinogenic, environmentally friendly, non-flammable, and easy to handle. In comparison to xylene, cedarwood oil demonstrated nearly superior performance in terms of nuclear and cytoplasmic staining across all tissue subgroups.[10,11,12] Effective clearing is crucial for achieving tissue translucency and visibility under a microscope, which is essential for accurate diagnosis.[13]

The findings of this study align with previous research indicating that cedarwood oil may be a more effective clearing agent than xylene, particularly in maintaining tissue clarity and uniformity. This suggests that essential oils could be a viable option for tissue clearing in histopathology laboratories.[14,15,16]

Although literature supports the use of cedarwood oil, results from various studies have been inconsistent.[17,18] This study demonstrated that an 8% cedarwood oil solution provided satisfactory staining in terms of clarity and uniformity. While cedarwood oil may be more costly compared to traditional agents, its safety benefits justify its consideration as a preferred clearing agent.[19]

CONCLUSION

Minimizing xylene use in histopathology laboratories is advisable without compromising processing accuracy and diagnostic quality. This study indicates that cedarwood oil can effectively replace xylene in routine histopathology, contributing to a healthier laboratory environment. The article was formulated according to SDG3 criteria of health and well-being. Despite promising results, further research with larger sample sizes is necessary to fully validate these findings.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical approval

The study was approved by the Institutional Ethics Committee and was conducted in accordance with the Declaration of the World Medical Association.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Source of funding

The work was not funded.

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