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Pathology

KEY WORDS: Xylene, Cedar wood oil, Coconut oil, Dishwash liquid (DWL)

A COMPARATIVE STUDY OF THE EFFICACY OF CEDARWOOD OIL, COCONUT OIL AND DISH WASH LIQUID AS ALTERNATIVES TO XYLENE AS DEPARAFFINIZING AGENTS

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ABSTRACT

Xylene is an aromatic hydrocarbon which is widely used as a deparaffinizing agent, and it is extremely biohazardous. Various biosafe alternatives to xylene have been studied in the past with variable results. The aim of this study was to compare the efficacy of Cedar wood oil, Coconut oil and Dish wash liquid (DWL) with Xylene as a deparaffinizing agent and to figure out the best biosafe alternative to Xylene. The study consisted of 50 samples and results were analyzed based on the cellular architecture and total quality of staining. Xylene and DWS showed same result of high-quality staining in case of cellular architecture. But in case of total quality of staining, only Xylene showed the best results. Hence, we could conclude that though Xylene is toxic, it is still the best deparaffinizing agent and more studies has to be done in this field to prove the efficiency of natural agents.

INTRODUCTION

Histopathology is an art of analyzing and interpreting the shapes, sizes and architectural patterns of cells and tissues within a given specific clinical background and a science by which the image is placed in the context of knowledge of pathobiology, to arrive at an accurate diagnosis.[1]

Hematoxylin and Eosin-stained Paraffin sections are the backbone of daily histopathological diagnostic work. Paraffin sections are conventionally prepared by methods largely unchanged for over 150 years. The process of deparaffinization of the slides using Xylene is an important preliminary step before the staining process, which makes the tissue sections to uptake the Hematoxylin and Eosin stain properly. This makes Xylene an unavoidable compound in histopathology due to its paraffin solvent action. But unluckily; Xylene is found to be a toxic compound, that is hazardous for human use and the environment in which it is disposed. Therefore, any substitute that minimizes the use of Xylene in experiments is the need of the hour.[2]

MATERIALS AND METHODS:

The present study consisted of 50 Formalin fixed paraffin embedded tissue blocks of previously diagnosed random cases which were collected from the departmental archives.

From each paraffin blocks, 4 sections of 3-4 µm were sliced using semiautomatic Microtome (MICROM model HM340E). Since sample size was 200, total 200 sections were obtained. Each section of same blocks was deparaffinized with Dish wash liquid, Coconut oil, Cedar wood oil and Xylene respectively at different time in a single day. Deparaffinizing agents were changed in a weekly basis to improve deparaffinization.

Table 1:- Deparaffinization & H and E staining procedure for Xylene, Cedar wood oil, DWL & Coconut oil:

Procedure	Material used	Temperature	Time
Deparaffinization	Xylene I	At room temperature	5 min
	Xylene II	At room temperature	5 min
	Cedar wood oil	At room temperature	4 hrs
		70°C	1 min
		Water wash with distilled water	5 min
	1.7% DWL	90°C	2 min
		Water wash with distilled water	2 min
	Coconut oil	90°C	2 min
		Water wash with distilled water	2 min
Rehydration	Descending grades of alcohol	At room temperature	10 min
	Water wash	At room temperature	10 min
Nuclear staining	Harri's Hematoxylin	At room temperature	8 min
	Water wash	At room temperature	2 min
Differentiation	1% acid alcohol	At room temperature	1 dip
	Water wash	At room temperature	10 min
Cytoplasmic staining	1% eosin	At room temperature	1 min
Dehydration	100% alcohol	At room temperature	5 min

The deparaffinized sections were stained with H & E and seen under Compound microscope. Assessment of staining was done by using the scoring system given by Sugunakar Raju Godishala et al.

- **Scoring system: -**

1. Cellular architecture:

1) SCORE 0: Indistinct nucleus - cytoplasm
2) SCORE 1: Distinct Nucleus – cytoplasm

2. Quality of staining:

1) SCORE - 0 = Poor
2) SCORE - 1 = Satisfactory
3) SCORE - 2 = Good[3]

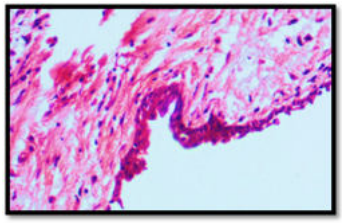


Figure 1: Deparaffinization with Xylene (H & E, 20X)

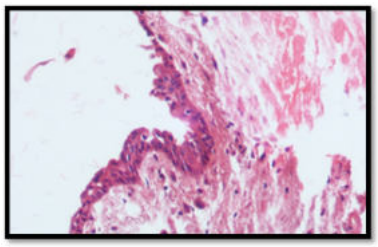


Figure 2: Deparaffinization with Dish wash liquid (H & E, 20X)

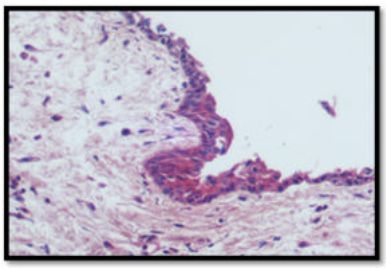


Figure 3: Deparaffinization with Cedar wood oil (H & E, 20X)

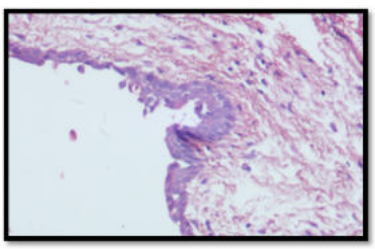


Figure 4: Deparaffinization with Coconut oil (H & E, 20X)

RESULTS

A total of 200 samples were obtained from 50 paraffin blocks and the results were tabulated and statistically analyzed using 'Chi-square test'. The test result is as follows;

I. Cellular architecture: As per the results, 100 % of Xylene and Dish wash liquid samples showed excellent cellular architecture and 74% of Cedar wood oil and 48% of Coconut oil samples showed excellent cellular architecture. In total from 200 samples 80.5% samples showed excellent cellular architecture. The result was statistically very highly significant with P value <.001.

ii. Quality of staining: As per the results, 92 % of Xylene and samples showed good quality of staining and followed by 42% of Cedar wood oil and 18% of DWL showed good quality of staining. Coconut oil showed the least quality staining. In total from 200 samples 40.5% samples showed satisfactory staining quality. The result was statistically very highly significant with P value <.001.

DISCUSSION

Deparaffinization is the procedure of removing paraffin wax from slides prior to staining. Since the paraffin wax melt at temperature around 70°C, an ideal deparaffinizing agent should be kept at 70°C for deparaffinization.

Choosing an appropriate deparaffinizing agent relays on the quality of stained sections, appropriate cellular architecture and time duration of procedure. The conventional method for deparaffinization is with Xylene.[4]

Xylene or "dimethyl benzene", is an aromatic hydrocarbon and it consists of a six-carbon ring to which two methyl groups are bound. It exists basically in three isomeric forms: ortho-, meta- and para-xylene and it is a colorless, sweet smelling liquid or gas occurring naturally in petroleum, coal and wood tar and it is named so because it is found in crude wood spirit (Greek xylon- wood). Xylene is commonly used in industry and medical technology as a solvent.[5]

The contents of laboratory-grade xylene are mainly; m-xylene, p-xylene, o-xylene and ethyl benzene and traces of toluene, thiophene, trimethyl benzene, hydrogen sulfide, phenol and pyridine.[6]

Xylene causes serious health issues when exposed. The main effect of inhaling xylene vapor is depression of the central nervous system, with symptoms such as headache, dizziness, nausea and vomiting. It can cause injury to the liver and kidneys if there occurs very high-level exposure and may have effects on gastro intestinal tract also. It is harmful on skin also, since, xylene can dissolve the skin's natural protective oils. Irritation is a main effect of xylene on skin. If there is no maternal toxicity, it may can cause fetotoxic effects like delayed ossification and behavioral effects in animals. Carcinogenic potential of Xylene is not reported till date.[5]

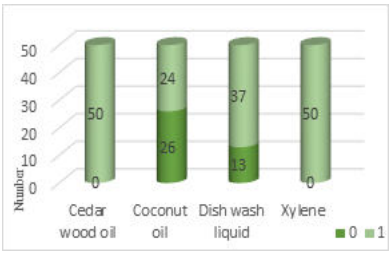
Substitution is the method of finding a substance that can perform almost the same function and which may lessen the hazard. The new substitute should not be hazardous in nature. Many potential substitutes became available after the hazardous effects of xylene became indisputable in the 1970s. In general, these substitutes can be categorized into four classes (but they are marketed under various company names) and are Limonene reagents, Aliphatic hydrocarbon mixtures, Aromatic hydrocarbon mixtures, Mineral oil mixtures. However, all these products have various advantages and disadvantages.[5] Hence, the present study was conducted to evaluate the efficacy of natural substitutes as alternative to xylene as deparaffinizing agents.

In the present study; the deparaffinizing agents which showed excellent Cellular architecture were Xylene and DWL. This was in accordance with the study conducted by Anuradha Ananthaneni et al[6] Madhuri R Ankle et al[7], Surekha Ramulu et al[8], Amita Negi et al[9], Pinki Pandey et al[10], Gayathri et a[12] , Anuradha Ananthaneni et at[8], where they concluded that DWS gives an excellent quality nuclear staining. Cedar wood oil and Coconut oil were less effective to assess cellular architecture compared to Xylene and Dish wash liquid. This was not in accordance with study conducted by Sudip Indu et al[11] and Ananthalakshmi Ramamoorthi et al[12]; where they got excellent result for Cedar wood oil and concluded that it can be an effective alternative to xylene. Coconut oil showed comparatively poor cellular architecture. Since there were no previous studies done on this same material; further studies can be done to acknowledge the actual efficiency of it.

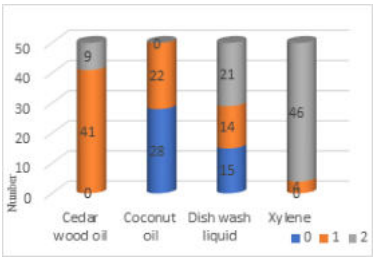
In terms of quality of staining; there was a very high significant difference between each agent. Excellent results were actually obtained with the hazardous Xylene and which was followed by Cedar wood oil and this was not in accordance with study done by Sudip Indu et al[11] and Ananthalakshmi Ramamoorthi et al[12], where they have found out that cedar wood oil have almost the same effectiveness as Xylene in deparaffinization to assess the quality of staining.

Dish wash liquid showed 82% of satisfactory result for quality of staining. But only 18% of them showed good quality staining. This was not in accordance with the studies done by Madhuri R Ankle et al[7], Surekha Ramulu et al[8], Amita Negi et al[8], Pinki Pandey et al[10], Gayathri et al[2] and Anuradha Ananthaneni et al[6]., where they found out that DWL showed excellent quality staining. They have found out that there is no significant difference between Xylene and DWL in terms of staining quality.

Graph 1:- Comparison of 'Cellular architecture':



Graph 2:- Comparison of 'Quality of staining



CONCLUSIONS

Based on the results obtained in the present study and the various other similar studies, we could conclude that though Xylene is toxic, it is still the best deparaffinizing agent for histopathology.

The limitation of the present study was mainly the staining quality assessment which was done by a single observer. Hence, more studies have to be conducted and analyzed by multiple observers and standardized protocol for the deparaffinization with natural substitutes has to be obtained.

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