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ORIGINAL ARTICLE

Comparing the use of Xylene and Cedarwood oil and its Efficacy in Hematoxylin and Eosin Staining. An Investigational Study

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ABSTRACT

Background: The use of xylene as a cleaning agent is required for hematoxylin and eosin staining. On the other hand, there is cause for concern when it comes to the dangers of xylene exposure. Xylene has been replaced with a variety of solutions, including essential oils, during tissue processing. The objective of this research was to see whether Cedarwood oil, an essential oil, could be utilised as a substitute for Xylene in Hematoxylin and Eosin staining.

Materials and Methods: The study was carried out in the Histopathology and Microbiology Department. The department's archives yielded thirty blocks of paraffin from the regular biopsy material. For my diffuser combination, I purchased cedar wood oil from an organic and natural goods store in my neighbourhood. The 30 tissue samples that were processed, each of the 30 tissue samples that was processed was washed with an essential oil (utilised 8 percent cedarwood oil) or xylene before being sliced into four-micron-thick slices and stained with E and H stain. They were rated based on the uniformity, clarity, and transparency of the stained sections.

Results: The three staining quality indicators tested showed a strong link between cedarwood oil and xylene.

Conclusions: It is our opinion that cedarwood oil may be used as a xylene substitute in the histopathology laboratory.

Keywords: H and E stain, xylene, histo-techniques,

INTRODUCTION

With a marvelous dewaxing and cleaning characteristics, xylene an aromatic hydrocarbon, is usually used for tissue staining (Kandyala, Raghavendra, Rajasekharan, & JOMFP, 2010)(Falkeholm, Grant, Magnusson, & Möller, 2001)

The gold standard for histological diagnosis is H and E staining. It is used to distinguish between the cytoplasm and the matrix, and it is quite stain in this regard(Kandyala et al., 2010)

Despite its value in histology staining, the use of xylene has been associated with workplace dangers. All of the body's major organs, including the skin, eyes, blood system, and nervous, are at risk. Because of its volatility and the fact that it cannot be contained completely, it may potentially contaminate the workplace(Falkeholm et al., 2001)

Hematology and pathology laboratories have previously attempted to limit or eliminate the use of the toxic chemical xylene by switching to limonene reagents and aromatic and aliphatic hydrocarbon alternatives as well as vegetable oils and edible oil replacements. (Kandyala et al., 2010)

It would also be good if xylene consumption could be reduced or eliminated, not only for diagnostic reasons, but also to provide a relatively safe laboratory atmosphere. A non-biohazardous substitute for xylene was sought in this study, and (8 %) cedarwood oil came out on top.

METHODOLOGY

The study was conducted at the Microbiology and Histopathology Department. Thirty blocks of paraffin were retained in the department's archives from routine biopsy specimens.Open market purchases were made for the essential oil (cedarwood oil) (Bon Appetite Go Organic outlet).Among all the 30 paraffinized normal tissues blocks, usually has two to three or four-micron-thick paraffin blocks. A mixture of xylene and cedarwood essential oil has been deemed essential oil After 4 hours at room temperature in an (8 percent) cedarwood oil solution, the cedarwood oil-cleared paraffin sections were microwaved for 1 minute at 60°C.

After cleaning slices in distilled water, a standard staining procedure of E and H was employed. The staining procedure of E and H was performed on sections that had been submerged in xylene overnight at 37-40°C, then each for 15 minutes subjected to xylene I and II. Cedarwood oil and protocol specificationsare summarised in these tables (1, 2). (xylene). Sections were evaluated based on graded based onstaining in the nucleus and cytoplasm (score 1=adequate, score 0=in adequate) as well as overall staining and staining uniformity. Each slide's score was then tallied. In order to be declared statistically significant, p-values of less than 0.05 were required.

RESULTS

Using cedarwood oil or xylene to clean the sections, 27 (90%) of the appropriate nuclear staining and 28 (93.3%) of the sections were seen respectively (Table 3). Table 3 shows that uniformity and cytoplasm of staining were both similar (about 94%) in sections washed with cedarwood oil or xylene. In addition, the outcomes were inconsistent (Table 4). We observed that the 30 sections cleaned with cedarwood oil had similar results in terms of the cytoplasm staining and sufficiency of nuclear as well as the overall uniformity of staining similar results were obtained (Figure 1).

Table 1:Deparaffinizing agent Cedar oil used in the Hematoxylin and Eosin Staining for Cleaning purpose

Steps	Solution	Temperature	Duration
Deparaffinization	Clearing agent Cedar wood oil	At room temperature	2-4h
Microwave Application	Clearing agent 60°C 1imin Washing In distilled water	At room temperature	5min
Rehydration Nuclear staining	100%,100%,100%, 80%,60% alcohol	At room temperature	2min each
Differentiation	Hematoxylin 2% Washing in running Tap water	At room temperature At room temperature	25min 5min
Dehydration	60%,80%,100% alcohol	At room temperature	2min each
Cytoplasmic Staining	1% eosin		
Dehydration	100%,100%,100% alcohol	At room temperature At room temperature	1min 5min

Table 2:Xylene as Deparaffinizing agent for Hematoxylin and Eosin staining as cleaning agent

Steps	Solution	Temperature-	Duration
Incubation	Overnight	37-40°C	
Deparaffinization	Clearing	At room temperature	15 min Each
Drying	Agentsixylene‑ I,II	At room temperature	30 min
Rehydration	100%,100%,100%, 80%,60% alcohol	At room temperature	2min each
Nuclear staining At room temperature	Hematoxylin Washing in running Tap water Acid alcohol At room temperature 2 dips Washing in running Tap water	At room temperature At room temperature Differentiation 2%	25 min 5 min 30 min
Dehydration	60%,80%,100% alcohol	At room temperature	2 min each
Cytoplasmic Staining	1% eosin	At room temperature	1 min
Dehydration	100%,100%,100% alcohol	At room temperature	5 min

Table 3: Association Between Cedar Oil and Xylene

Staining characteristics	Adequacy N(%) Cedarwood Xylene N% N %	P-value on Chi- square test
Nuclear staining	27 90 28 93.3	0.640
Cytoplasmic staining	28 93.3 29 96.7	0.554
Uniformity	2 93.3 29 96.7	0.55

Table 4:Cedar oil comparison with xylene

Grading Adequate In adequate	Cedarwood oil Xylenei N% N %	P-value on Chi- square test	P-value
	8291.118695.55 80.88 4 0.44	1.429	0.233

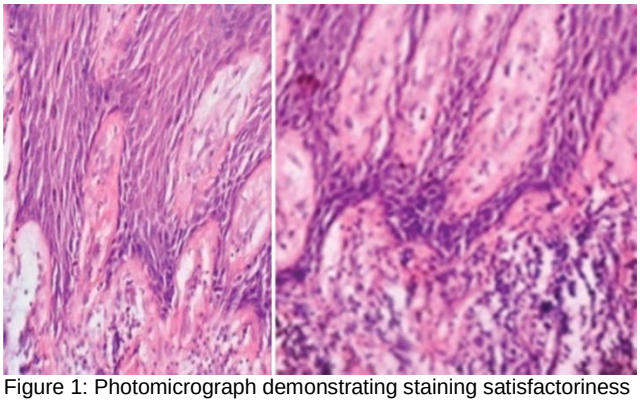

Figure 1: Photomicrograph demonstrating staining satisfactoriness and homogeneity. (a) The area cleared with cedarwood oil. (b) The part that was cleared with xylene (H and E, 40).(c)

DISCUSSION

In histology laboratories, xylene has been a vital component for the last six decades, offering an effective alternative to more toxic cleaning chemicals like benzene and chloroform. However, xylene is flammable and may cause skin irritation, heart and blood vessel problems, as well as neurological or renal damage(Miller, Miller, Driscoll, & Miller, 1994).

As xylene is a highly flammable material, it is difficult to dispose in laboratories where it is often used. As a result, we decided to look at essential oils (such as cedarwood oil) as possible cleaning agent alternatives in our study. Using essential oils is safe since they are nontoxic, biohazardous, and environmentally friendly. Previous research has shown that cedarwood oil is a superior cleaning agent than xylene in tissue processing, and our results confirm this (perfect ribboning) (Indu et al., 2014; Premalatha, Patil, Rao, & Indu, 2013).

Prior to impregnation and embedding of wax and tissue essential oils maybe used in the stage processing of tissue in order to achieve the same results. As a natural substance, cedarwood oil has a wide range of traits and characteristics. In the literature, histological processing needs a minimal amount of fluid diversity (Körbler, Gršković, Dominis, Antica, & pathology, 2003). The time it takes for oil to clear depends on its viscosity; less viscous oils take shorter to clear than viscous oils. The production of practically minimal tissue damage is one of the beneficial key of this oil. It does, however, take a lot longer to process and is significantly more costly than the other options (Indu et al., 2014)

However, utilising the best procurement practises may help to offset the higher costs. Furthermore, it has been shown that tissue cleansed with cedarwood oil is substantially less likely to discolour (Panda, 2005)having valuable property of clearing the tissue, Cedarwood oil consistently had good staining uniformity, which show similarity with the results obtained, on the other hand cleared tissue sections of cedarwood oil consistently ensuredutmost valuable uniformity staining due to its mild and non-destractive effects on tissues.

Even while a water solution of common dishwashing detergent has shown potential as a alternative for the toxic tissue segment cleaning of xylene, its probable effects on certain tissue structures remain a concern(Ankle, Joshi, & JOMFP, 2011).Our work used chemically ripened Ehrlich's hematoxylin, rather than Mayer's hematoxylin, to stain the nuclear components (Ankle et al., 2011; Falkeholm et al., 2001)

Since Ehrlich's hematoxylin has been chemically ripened, it has a longer shelf life and hence better staining quality. In spite of this, Harris' hematoxylin, which is widely used in Indian laboratories, has the potential for both progressive and regressive uses. (Indu et al., 2014)Clearing agents like lemon and cedarwood have been extensively studied. (Bancroft & Gamble, 2008).Tissue processing research employing these solutions has shown inconsistent results.

At least 8 percent cedarwood oil produced noticeable tissue discoloration in terms of transparency and homogeneity in this experiment, despite its higher cost. These plant oils should not be used to cleanse or alter tissue because of potential safety issues.

CONCLUSION

Using essential oil (8%) E and H staining was found to provide satisfactory results in this study, with suitable clarity and uniformity. Moreover, it is non-flammable, non-hazardous, and non-toxic in addition to being simple to use. A long-term evaluation of tissue staining durability in E and H stained (xylene free) sections is still necessary, however.

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